Physical Properties of Simple and Complex Sugar Systems

Balaji Subbiah

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Department of Chemical & Process Engineering

University of Canterbury

Christchurch, New Zealand

Dedicated to my sisters

Swarnalakshmi &

Sivasankari

ABSTRACT

Honey, maple syrup and sugars during refining are examples of a complex solutions of concentrated sugars and other solutes. The physical properties of such systems are required for the design and optimization of processes and for the understanding of the behaviour of the final product. Literature shows limited data and even less rigorous analysis of the physical properties of concentrated sugar solutions. A fundamental thermodynamic approach provides an effective basis for the analysis and prediction of these properties. The main focus of this study was to consolidate physical property data for binary sugar systems, analyse the data using existing models, and propose newer models and equations to predict the physical properties of complex sugar solutions like honey.

In order to approach this, the physical properties density, viscosity, electrical conductivity, refractive index, mutarotation and water activity of binary solutions and sugar mixtures ranging from invert sugar solutions to model honey solutions with acid and salt were measured. Models that have been applied to sugar systems were reviewed. An equation for density based on the apparent densities of sugars in solution was validated. A viscosity equation that was extended from previous work with concentrated milk systems, was found to be very effective over the full concentration range of complex sugar solutions. Effects of viscosity on the electrical conductivity of sugar systems were analysed and a model was proposed for electrical conductivity in sugar systems. Hydration theory based on water activity measurements was applied to binary sugar systems and extended to model honey systems. Mixing theories were applied to the calculation of refractive index of solutions and also used as a tool to confirm the solid mass fraction in solutions.

The effect of mutarotation of sugars in viscous sugar solutions was studied using a new NMR technique that was validated using polarimetric measurements. In this study it was found that the mutarotation of glucose was not affected by the viscosity of the solution.

Past and new data and resulting equations provide a solid basis for future modelling and simulation of crystallization in these systems.

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I thank my parents for their support in providing me a quality education and respect all their sacrifices. Next, my sisters, who help me get my education at the cost of their own due to my family's financial circumstances. A special thanks to all my friends till date.

My supervisor Associate Professor Dr. Ken Morison has been a pillar of strength and I thank him for his ideas and guidance. Dr. Morison is a real inspiration and has always treated me as an equal and never as a student. I would have to make a note about his patience, keeping in mind my slow work ethic and performance. He has made me evolve as an individual with ability to think, problem solve and equip me with better work ethics. If I can summarize, he is a real "guru" and honestly has become my family in the process.

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Finally, I conclude by giving thanks to the positive energy above for all the opportunities.

PREFACE

My inspiration to obtain a higher education degree was derived from the quote that follows below which means, learning is the true imperishable riches; all other things are not riches.

கேடில் விழுச்செல்வம் கல்வி யொருவற்கு
மாடல்ல மற்றை யவை[1]. - Thirukkural (400)

Technology is the application of knowledge and understanding of science to the development of more economical and convenient methods for recovering useful materials from nature to use. The work presented in this thesis shall contribute to fundamental understanding of thermodynamic properties of food systems with sugars by revealing one or more of the characteristic physical properties.

The work mentioned in the thesis was performed at the Department of Chemical & Process Engineering, University of Canterbury between August 2014 and July 2018. The experimental work discussed in this thesis was developed by me and my supervisor Assoc. Prof. Ken Morison. My supervisor has been an inspiration throughout the duration of the course of my study.

[1] The text is written in an ancient language known as Tamil. The quote is 400th among the 1330 quotes which were compiled into a book by the poet, Thiruvalluvar who lived in the 14th century BC. Thirukkural is a book giving detailed insight into leading a human life.

“Try to be a person of value instead of looking to be a successful person. Success is a consequence.”

- Albert Einstein

PUBLICATIONS

1. Subbiah, B., Morison, K. R., **2018,** Electrical Conductivity of Viscous Liquid Foods, *Journal of Food Engineering*, 237, 177-182.
2. Subbiah, B., Blank, U., Morison, K.R., **2018,** Fundamentals of Water Activity in Honey. (Manuscript ready for submission).

Conferences

3. Subbiah, B., Morison, K. R., **2018**, *Effect of sugar ratios, salts, and acids on physical properties of complex sugar systems that contribute to crystallization.* Thanjavur, India: International Conference on Recent Advances in Food Processing Technology (iCRAFPT), 17th -19th August 2018.
4. Subbiah, B., Morison, K.R., **2017**, *Effect of viscosity on electrical conductivity in liquid foods.* Melbourne, Australia: CHEMECA Conference, 26th July 2017.
5. Subbiah, B., Morison, K.R., **2016**, *Physical Property Equations for Model Honey Solutions and their Application to Real Honey*. Bangkok, Thailand: Proceedings of the 2016 International Conference on Food Properties (ICFP2016), May 31-June 2, 2016.
6. Subbiah, B., Stembridge, A.L. and Morison, K.R., **2015**, *Measurement and Calculation of the Electrical Conductivity of Model Honey Solutions*. Melbourne, Australia: APCChE 2015 Congress, 27 Sep - 1 Oct 2015. (Peer-reviewed Conference Contribution - Published).

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LIST OF ABBREVIATIONS

a_s sugar activity

a_w water activity

D_i diffusion coefficient

e_0 charge of an electron

Δh_f latent heat of fusion

Δh_v latent heat of vaporisation

k_b Boltzmann's constant

m molality

M molecular mass

$\bar{n}$ hydration number

n refractive index

R universal gas constant

r_i Stokes' radius

T_g glass transition temperature

$\bar{V}$ molar volume in m^3/mol

w mass fraction

x mole fraction

z ionic charge

η viscosity Pa·s

η_r relative viscosity

γ activity coefficient

μ chemical potential

Λ_m molar conductivity

ϕ volume fraction

ρ density in kg/m^3

ρ_w density of water in kg/m^3

ρ_s apparent density of sugar in kg/m^3

ρ_{mix} density of the mixture in kg/m^3

σ electrical conductivity in µS/cm

v kinematic viscosity

1 INTRODUCTION

1.1. Overview

Food research has taken a huge leap forward in the last century. Numerous techniques have been developed to quantify and qualify food for specific uses. These methods and techniques have helped to increase shelf-life of foods; identify the correct treatment parameters for different types of foods; define colours, textures, shapes, sizes, tastes, states and so on. A better understanding and knowledge of the thermodynamic properties of food materials helps to design and develop better production methods and has paved the way for further research. Thus, understanding of properties of food materials is very useful.

As an example, the freezing point depression, osmotic pressure, boiling point elevation and water activity of liquid food are all related through the mole fraction of water and its activity coefficient, but seldom are all these properties treated as one. These thermodynamic properties help us to understand the interactions between the solute and the solvent present in the solutions. The main aim of this project was to find opportunities to extend experimental data, theory, and models to be better able to predict these properties. In order to achieve the goals that are set out, large quantities of reliable and accurate experimental data are required of samples that would be considered for the work. Most physical property data were measured at different concentrations, temperature, frequencies and other parameters depending upon the property being measured. There are opportunities to attempt and apply better correlations to existing data as well as obtaining newer data. Much of the necessary theory already exists but has not been applied to foods.

As a starting point, the properties of mixtures of concentrated solutions of sugars and salts were measured to determine behaviour in solutions where there is competition for hydration water. Models proposed in the literature are discussed in detail and proposed in this work to enhance understanding. Sufficient experimental data were accumulated to substantiate the theories proposed in this work. This has led further into the studies of crystallization of sugars such as glucose in concentrated systems (Starzak & Mathlouthi, 2002).

1.2. Food Systems with Sugars – A Background

Sugars contribute to many major foods in today's world. Some of the high sugar foods range from simple sugars like sucrose, fructose, and glucose to complex sugar systems like fruit concentrates, honey, milk, and maple syrup. These sugars are available as crystalline powders or syrups with mixed sugars (Jackson & Silsbee, 1924; Scatchard, 1921).

Milk naturally contains approximately 48 g of lactose in 1 L of whole milk (McCarthy & Singh, 2009). Properties like hydration, surface activity of proteins in milk are affected by sugars thereby altering the stability of systems derived from milk like sweetened condensed milk, sweetened yoghurt and others (Gao et al., 2010). Collection of data and understanding of the effect these sugars in a food system like milk will help improve processing conditions during the manufacture of different products (Sharifi & Young, 2012). Sweetened condensed milk is a commercially available evaporated dairy product which has nearly 55% lactose + sucrose mass basis and sucrose crystals are visible in the microscopic images (Tamime, 2008).

Honey is a natural complex sugar system with a mixture of sugars including glucose, fructose sucrose, other sugars, salts and acids. The composition of honey depends on its source and type, eg. floral or honeydew honey. As this study aims to understand the complexities in complex sugar solutions, a general understanding of the constituents of honey is essential. Honey is in general characterised using sensory analysis and chemical analysis (Guo et al., 2010). Table 1 shows the comparison of compositions present in a typical honey sample and milk. Honey is a highly concentrated sugar solution (Zamora & Chirife, 2006). Sugars represent the largest portion of up to 95% to 99.9% of solid honey composition (White Jr. & Doner, 1980), with around 80% to 85% of this being the monosaccharides fructose and glucose. Glucose accounts for 33% to 35% of solid honey, while 38% to 40% is fructose (Doner, 1977). Smaller quantities of disaccharides, such as sucrose and maltose, acids, proteins, vitamins, pigment, and aromatic substances also occur in honey (Zamora & Chirife, 2006). Since the concentrations of these components are so small in comparison to the proportion of sugar present, honey is expected to have very similar properties comparable to a model solution of water, fructose and glucose. Glucose syrups and honey are demonstrated to be used as model food systems and models for geological studies as viscous behaviour is predominant (Schellart, 2011).

Table 1 Typical composition of Milk (Walstra et al., 2006) & Honey composition range (White et al., 1962)

Milk		Honey	
Component	Mass fraction	Component	Mass fraction range
Water	87.1%	Water	13.4% -17.2%
Solids-not-fat	8.9%		
Fat in dry matter	31.0%		
Lactose	4.6%	Fructose	27.2% - 44.3%
Fat	4.0%	Glucose	22.0% - 40.7%
Protein	3.3%	Maltose	2.7% - 16.0%
Casein	2.6%	Sucrose	0.2% - 7.6%
Mineral substances	0.7%	Higher sugars	0.1% - 8.5%
Organic acids	0.17%	Ash	0.02% - 1.028%
Miscellaneous	0.15%	Other/Undetermined	3.2%

Variations in the exact sugar and minor component compositions in honey highly depend on the regional and climatic conditions, flower source and type of raw material used by the bee (nectar or honeydew) (Al-Habsi et al., 2013). Although sugars are the principal factors which determine the physical attributes and behaviour, it is the minor components that are responsible for any differences between honey types (White Jr. & Doner, 1980) including colour, flavour and aroma. Floral and honeydew honeys have varying compositions of both minor and major sugar components because of their raw material characteristics.

Table 2. Average Composition of Maple Syrup (Ball, 2007).

Components	Mass fraction
Sucrose	68%
Glucose	0.43%
Fructose	0.30%
Water	31.7%
Malic acid	0.47%

Maple syrups produced from sap of naturally occurring maple trees has nearly the maximum solubility level of sucrose along with water and traces of glucose, fructose and other trace minerals and acids. Table 2 shows the typical composition of maple syrup (Ball, 2007; Takano, 2005) measured and summarised some of the physical properties of maple syrup. However, more comprehensive studies are needed to understand these food systems.

Understanding the physical properties of complex sugar systems like honey, milk, maple syrup is very useful in order to design and modify the existing process conditions of processing and also develop fundamental relations and models (Subramanian et al., 2007; Yanniotis et al., 2006). Hence, the significance of estimation of these properties will have a noteworthy impact on product design also. Lack of information about the physiochemical properties of honey was the initial driving force for this work.

1.3. Currently measured properties of honey

Over the years, data for the physical properties of foods have been well documented. However, while most of the data available are of excellent quality, the physical property data available for milk, honey and other foods are of mixed quality. Knowledge of the interaction between properties can help decide the best property to make measurement and also test consistencies in measurements (Williamson, 1967). Some commonly measured physical properties of solutions to understand thermodynamic relations are density (ρ), viscosity (η), water activity (a_w), moisture content, surface tension (γ), pH, conductivity (σ), dielectric constant (ε'). Though most of the properties above are reported in the literature, some properties have gained predominance.

The composition of a material contributes to almost all physical properties and in turn to the measured quality of honey samples. These include moisture/water content, electrical conductivity, mineral (ash) content, hydroxymethylfurfural (HMF), sugar contents and acidity (Bogdanov, 2009; Codex, 2001). Dielectric studies, viscosity, density, refractive index, water activity, crystallization parameters are some of the other properties that are measured for binary, ternary, complex sugar and honey solutions. Sensory analysis techniques are also being used in the industry to measure some properties and detect adulteration.

1.3.1. Density

Density is one of the fundamental physical properties that has been measured for sugars since the eighteen hundreds. Density of binary sugar solutions of sucrose, lactose were measured and reported in literature since 1900 and over the years, sufficient data on sugar systems have been reported (Barber, 1965; McDonald & Turcotte, 1948; Schwers, 1911; Snyder & Hattenburg,

1963). Model food systems with a mixture of solutes were studied and documented (Comesaña et al., 2003; Ruddick & Shirtcliffe, 1979; Vázquez et al., 1995). The typical density value of honey ranges from 1380 to 1450 kg/m^3 at 20 ºC (Tomasik, 2004). Density helps us in understanding the simple molecular relations as well, and sufficient density data is available in the literature that has been interpreted in numerous ways.

1.3.2. Electrical Conductivity

The electrical conductivity of binary, tertiary and complex sugar solutions are scarcely available. (Zerban & Sattler, 1931). According to Codex 2001 standards, electrical conductivity of honey should not be more than 0.8 mS/cm when measured at the required dilution (when 20 g of honey solids is diluted to 100 mL with water). Electrical conductivity of honey measured by the Codex standard 2001and the ash content in the samples are linearly related to each other (Kropf et al., 2008). The current work focuses on extension of intrinsic factors like viscosity that affect the measurement of electrical conductivity in these complex food systems.

1.3.3. Viscosity

Like density, there is much data available for viscosity of concentrated solutions such as concentrated milk, but the current correlations do not fit all the data well (Morison et al., 2013). Once the total solids content of milk gets beyond about 50% it becomes very viscous and gels easily. However, in some cases it is desirable to process the products as fluids. Models are required to explain changes in viscosity with concentration and time to enable better prediction and understanding of flows in process equipment. In this work, viscosity effects on other properties and food systems like milk and model honey are compared. The aim is to advance our understanding of molecular phenomena based on these properties. Both water activity and viscosity in concentrated fluids are controlled by the availability of water. While this has been explored for concentrated sucrose solutions by Starzak and Mathlouthi (2002) there are many opportunities to extend the ideas to complex mixtures.

1.3.4. Refractive Index

Refractive index usually is used to measure the moisture content in solutions. As the refractive index of sucrose solutions are well documented, refractometers are sometimes calibrated with sucrose solutions. Refractive index is usually measured for honey samples to confirm the moisture content (White Jr. & Doner, 1980). Similar to earlier work, this work will also use refractive index as a tool to confirm the solids content in the prepared solutions. The refractive index mixing model used in Reis et al. (2010) is also tested with the prepared solutions.

1.3.5. Water Activity

Water activity is another property that is getting prominence in understanding food properties. The water activity data for binary and ternary sugar solutions are available aplenty (Bhandari & Burel, 2007; Gleiter et al., 2006; Starzak et al., 2000). However, these data needs to be analysed and models need to be developed as aimed in this work. Water activity is now a tool in measuring microbial activity and shelf life of mid-to-low moisture content foods (Ergun et al., 2010). Honey is a complex mixture of sugars for which the prediction of water activity is little understood (Seow & Teng, 1981; Chirife, Fontan, & Benmergui, 1980; Fysun et al., 2015). Starzak et al. (2000) used hydration of sucrose solutions to establish the understanding of water activity of these systems. An emphasis is made to establish a relation on the basis of hydration to understand these model food systems from the measured data.

1.3.6. Dielectrics

The dielectric properties of honey is a measurand that is not included in the harmonized methods of International Honey Commission. However, considerable amount of research into sugar solutions have been compiled in the last two decade and data are available (Bordi et al., 2004; Ikeda et al., 1997; Liao et al., 2001; Malmberg & Maryott, 1950).

1.4. Research Objectives

Although, most of the properties have been measured and reported in the literature, this work further enhances the data and understanding by proposing newer models and analysis. The

results obtained for properties measured like density, viscosity, refractive index, electrical conductivity, water activity are discussed with models explaining the same. A part of the focus was to develop a credible model for crystallization in systems like honey.

The research objectives are:

- Compare and consolidate physical property data for binary to complex sugar solutions like honey.
- Measure the physical properties of model honey solutions and apply these to variable natural honey.
- Identify the influence of water in each physical property and summarize the effects on honey and its solutions.
- To apply better correlations to the existing data as well as generating new data.
- Extend experimental data, theory, and models to be able to predict these properties better.
- Determine relationships that will be useful for a range of mixtures.

The document if further organised into chapters of literature review, materials and methods followed by results and discussions. Finally, relevant conclusions and observations for future studies are made in the respective sections.

2 LITERATURE REVIEW

The physical properties of simple and complex sugar systems are discussed in detail in this review. In the process, conscious efforts were made to understand the effect of water in these complex solutions and its interactions with sugars. Further, the scope of this review is to understand some physical properties. This chapter starts by discussing the chemistry behind the carbohydrates in this study and then details the literature available on the physical properties.

2.1. Overview

A typical diet requirement of an adult consists of carbohydrates, proteins, fat, water, vitamins and minerals. Most foods contain a combination of these components. Major sources of carbohydrates are rice, wheat and fruits. Proteins and fats are obtained from milk, egg, meat and oil. Carbohydrates, which are the major source of energy, have the following accepted classification as found in Table 3 by the Food & Agriculture Organization in 1998. The name saccharides arrive from Latin for sugar (origin = "sweet sand").

Table 3. Carbohydrate Classification (Cummings & Stephen, 2007).

Class (DP[a])	Subgroup	Principal Components
Sugars (1-2)	Monosaccharides	Glucose, fructose, galactose
	Disaccharides	Sucrose, lactose, maltose, trehalose
	Polyols (sugar alcohols)	Sorbitol, xylitol, mannitol, lactitol, erythritol, isomalt, maltitol
Oligosaccharides (3-9) (short-chain carbohydrates)	Malto-oligosaccharides (α-glucans)	Maltodextrin
	Non-α-glucan oligosaccharides	Raffinose, Stachyose, fructo and galacto oligosaccharides, polydextrose, inulin
Polysaccharides (≥10)	Starch (α-glucans)	Amylose, amylopectin, modified starches
	Non-starch polysaccharides (NSPs)	Cellulose, hemicellulose, pectin, arabinoxylans, β-glucan, glucomannans, plant gums and mucilages, hydrocolloids

[a] Degree of polymerization or number of monomeric (single sugar) units. Based on Food and Agriculture Organization/World Health Organization 'Carbohydrates in Human Nutrition' report (1998), and Cummings et al. (1997)

2.2. Sugars and Basic Chemistry

As in Table 3, sugars are classified as monosaccharides, disaccharides and other higher sugars as oligosaccharides. Monosaccharides are the simplest unit of carbohydrates which are colourless, water-soluble and crystalline solids. In other words monosaccharides are the monomers for the other polymer carbohydrates like di-, tri- and oligo-saccharides. Glucose and fructose are monosaccharides which combine to form sucrose, a disaccharide while lactose is a milk protein contains glucose and galactose monomeric units. Figures 1 and 2 show some common mono- and disaccharides. Here, 'D' denotes dextrorotation of the sugar.

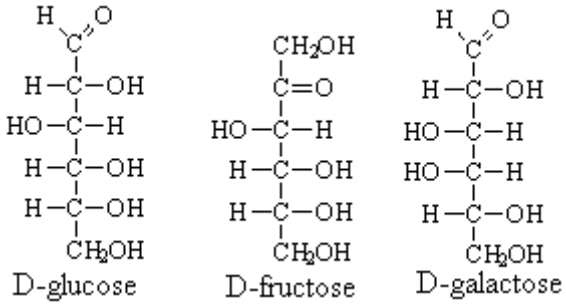


Figure 1. Structure of monosaccharides.

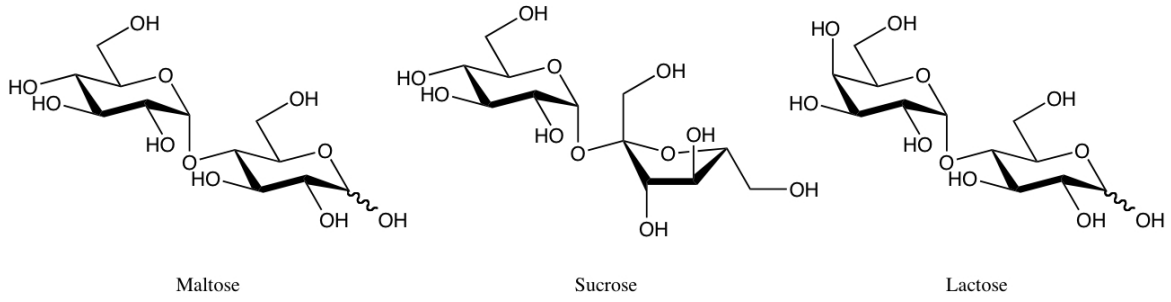


Figure 2. Disaccharide structures.

Monosaccharides are also called simple sugars while disaccharides are referred to as compound sugars. Mono and di-saccharides contains ketone (R-O-R') or aldehyde (R-CHO) functional groups in their chemical structure. Glucose has an aldehyde functional group, so belongs to a family referred to as aldoses while, fructose has a ketone functional group so is known as a ketose. Fructose has a higher solubility than glucose and sucrose at any given temperature probably due to its functional group.

Glucose and fructose exists in both α and β forms. In solutions both these sugars mutarotate to form different isomers in equilibrium. These different forms, known as isomers, have the same chemical formula but different structural orientation of atoms in the molecule. The isomers in equilibrium are called tautomers. Some of the possible structural tautomers of fructose are α-fructopyranose, β-fructopyranose, α-fructofuranose and β-fructofuranose (Cockman et al., 1987; Flood et al., 1996; Shallenberger, 1978). Some the glucose tautomers in solution known are α-glucopyranose and β-glucopyranose (Nelson & Beegle, 1919; Silva et al., 2006).

Sucrose in solution undergoes hydrolysis reaction in the presence of acids or enzymes and gets converted into monosaccharides glucose and fructose. The disaccharide sucrose splits up into a 1:1 mixture of the monosaccharides fructose and glucose Thus an equal mixture of glucose and fructose is referred to as invert sugar. The presence of one sugar type decreases the solubility of the others as investigated by Jackson and Silsbee (1924), Kelly and Brown (1967).

The model food system under primary investigation is thus a combination of mono- and disaccharides. A system that includes some of these sugars like glucose, fructose, and sucrose is hence chosen as the topic of investigation.

2.3. Types of solutions

An illustration of the types of solutions that will be dealt in this work is as follows. A binary solution is one that contains one solute (sugar) and one solvent (water). Ternary solutions is one that has more than one solute and a solvent or sometimes more than one solvent. A typical representation of solutions with various sugar concentrations ranging from low concentration to high concentration is shown in Figure 3. Typically solutions with low to medium concentration, exhibit ideal behaviour while solutions with high sugar concentrations exhibit non-ideal behaviour.

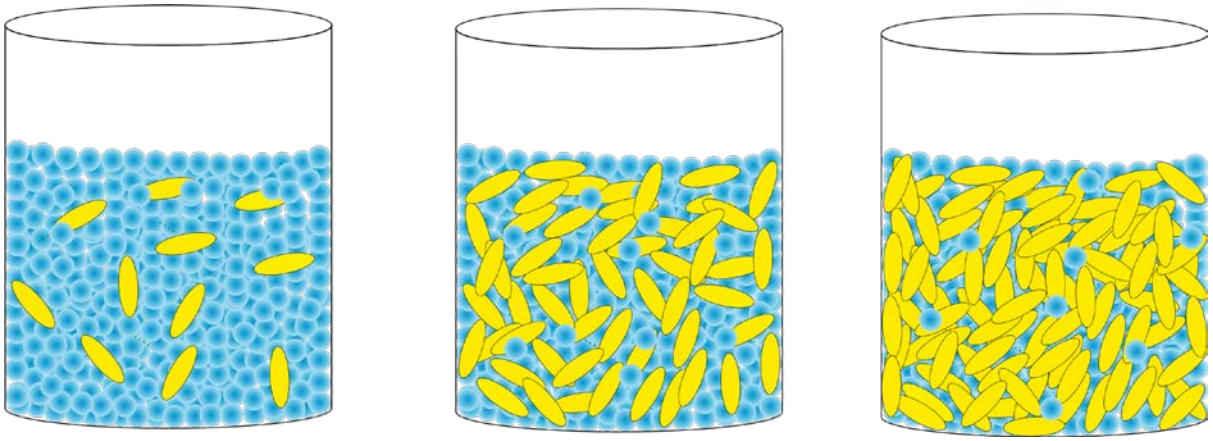


Figure 3 Illustration of solutions with one solute (sugar in yellow) and solvent (water in blue) and various concentrations. From the left to right, low, medium and high sugar concentration.

Figure 4 illustrates a typical multicomponent system and can also be referred as a complex sugar system. It is a representation of a model honey system similar to the ones that will be used in this work. This illustration will help understand the properties discussed in this review. Glucose and fructose similar in structure, are represented by the oval shaped molecules in bright yellow colour. Sucrose being the disaccharide is shown in an oval shaped molecule with pale yellow colour. The red coloured oval shape represents the ions from the acid and the black squares represent the ions of the salts.

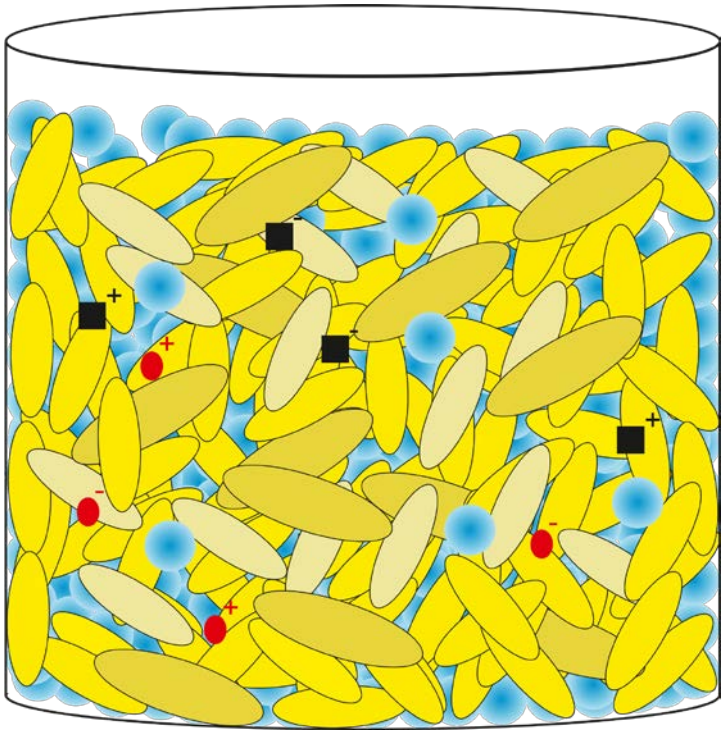


Figure 4 A model honey illustration with glucose (bright yellow ovals), fructose (bright yellow ovals), sucrose (large pale yellow ovals), salts (red) and acids (squares) (multicomponent solution).

2.4. Physical Properties

The physical properties of sugar solutions that are discussed here are as follows: density (ρ), viscosity (η), water activity (a_w), moisture content, surface tension (γ), pH, and conductivity (σ). Table 4 summarises the types of sugar systems and the properties that were being investigated and it gives an idea of the importance of this work.

The fundamental approach to measure these physical properties is that they depend on the shape, size, structure, behaviour as a solution and contribute to the understanding and development of fundamental relationships. The understanding that most of these properties are interdependent and the possibility of understanding these relationships has been a constant motivator for this work.

Table 4 Measured physical properties on sugar systems.

Property	Year	Sample	Author
Viscosity	2014	Honey	Santos et al. (2014)
	2013	Sugar solutions (mono-, bi-& tri-saccharides)	Ali et al. (2014)
	2009	Apple juice	Benı´tez et al. (2009)
	2009	Crystallized honey	Chen et al. (2009)
	2009	Honey	Gomez-Diaz et al. (2009)
	2007	Sugar solution (binary)	Telis et al. (2007)
	2007	Sugar solution (ternary & quaternary)	Yansheng et al. (2007)
	2006	Sugar solution (binary)	Cummins et al. (2006)
	2006	Sugar solution (binary)	Quintas et al. (2006)
	2006	Honey	Recondo et al. (2006)
	2003	Sugar solution (ternary)	Comesaña et al. (2003)
Density	2014	Sugar solutions (mono-, bi-& tri-saccharides)	Ali et al. (2014)
	2012	Sugar solution (binary)	Simion et al. (2012)
	2008	Sugar solutions (mono and di saccharides)	Gharsallaoui et al. (2008)
	2007	Sugar solution (ternary & quaternary)	Yansheng et al. (2007)
	2003	Sugar solution (ternary)	Comesaña et al. (2003)
	1998	Sugar solution (invert)	Bettin et al. (1998)
	1965	Sugar solution (binary)	Barber (1965)
Water activity	2017	Sugar solutions (binary)	Maneffa et al. (2017)
	2014	Honey	Shafiq et al. (2014)
	2010	Sugar solutions (binary)	Baeza et al. (2010)
	2008	Honey	Abramovic et al. (2008)
	2006	Honey	Chirife et al. (2006)
	2006	Honey	Zamora and Chirife (2006)
	2008	Sugar solutions	Gharsallaoui et al. (2008)

	2006	Sugar solutions	Starzak and Mathlouthi (2006)
	2003	Sugar solution (ternary)	Moreira et al. (2003)
	2001	Sugar solutions	Mathlouthi (2001)
	2000	Sugar solutions (binary)	Starzak et al. (2000)
Refractive index	2014	Sugar solutions (binary)	Ali et al. (2014)
	2010	Binary mixtures (theory)	Reis et al. (2010)
	2007	Sugar solutions (binary)	Fucaloro et al. (2007)
	2000	Sugar solutions (ternary)	Flood and Puagsa (2000)
	1981	Sugar solutions (binary)	Agriculture (1981)
Electrical conductivity	2012	Honey	Gómez-Díaz et al. (2012)
	2011	Honey	Guo et al. (2011)
	2011	Honey	Kaskoniene et al. (2010)
	2009	Sugar solutions (ternary)	Longinotti and Corti (2009)
	2008	Sugar solutions (binary)	Corti et al. (2008)
	2007	Honey	Acquarone et al. (2007)
	2004	Honey	Szczesna and Rybak-Chmielewska (2004)
	2004	Sugar solutions (ternary)	Longinotti and Corti (2004)
	2000	Sugar solutions (ternary)	Miller et al. (2000)
	1987	Honey	Accorti et al. (1987)

For each property the best equations will be obtained if the appropriate basis for concentration can be determined. For example electrical conductivity is based on the molar concentration of ions in solution, whereas refractive index has been shown to be a volumetric relationship. Viscosity seems to be related to the amount of water, and water activity for mixtures is much better expressed with the help of available water for hydration of the sugars. This dependence of each property to the most appropriate basis or bases is aimed at.

The principle behind the research is to connect the physical properties measured and correlate these to some thermodynamic properties like depression in freezing point, elevation in boiling point and so on. The base for this can be seen as shown for a binary solution by Reis et al. (2010). Neelwarne (2013) discussed how these measured physical properties can be correlated as it is currently done in the scale up of bioreactors. Figure 5 describes a collection of inter-dependence of some of the physical properties and is used as a starting tool/indicator for further understanding. Most of these inter-dependencies will be discussed as part of the individual property review in this section and further in the results and discussions.

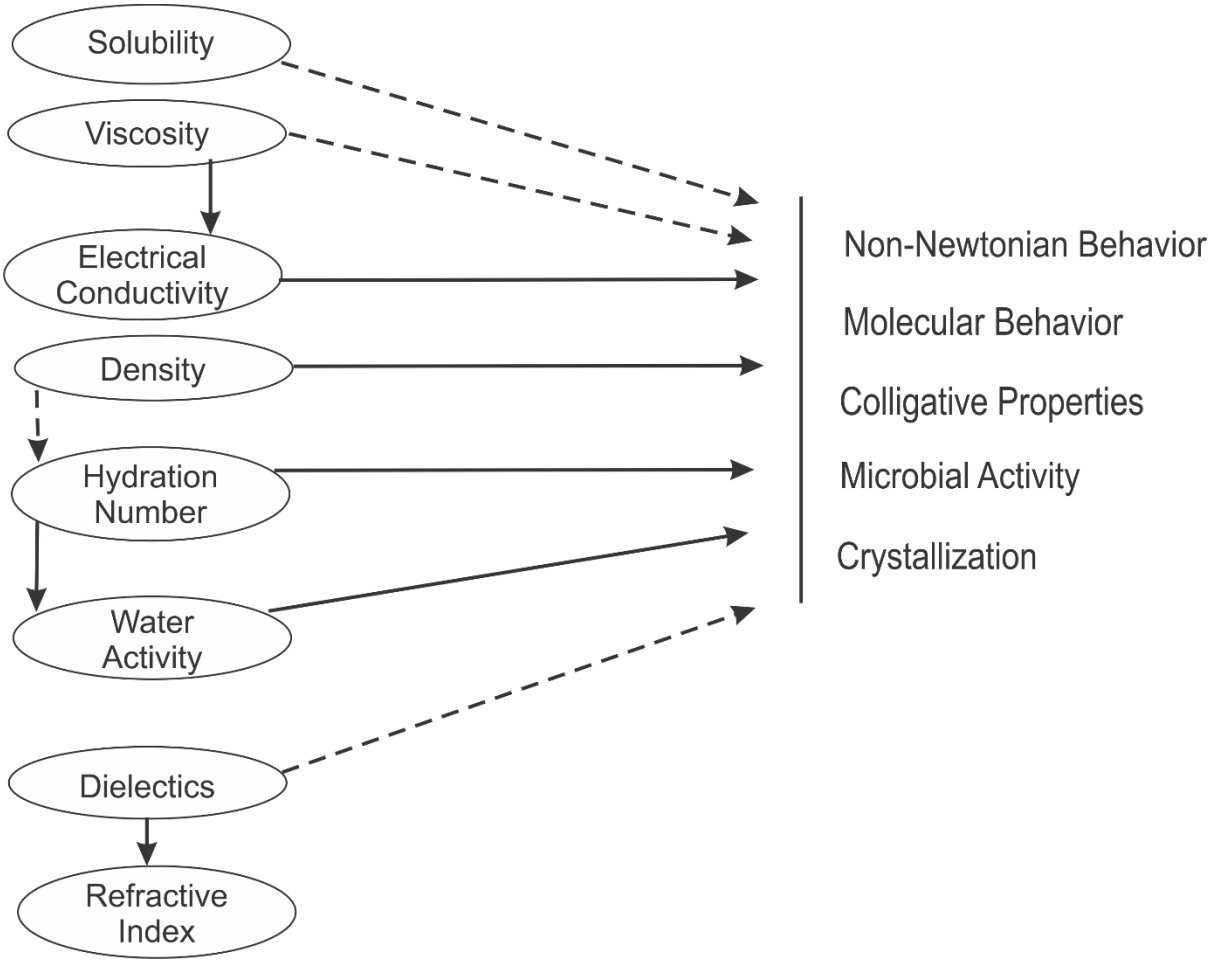


Figure 5 Indicator of the inter-relations of physical properties of food system. The dashed lines indicate weak or indirect interactions; the solid lines indicates direct or strong interactions within a food system.

2.3.1. Density

All literature data for density is in, or converted to, kg/m^3. The density of a mixture of solutes is best calculated as the mass fraction weighted apparent volumes, which can be expressed as:

$$\frac{1}{\rho_{soln}} = \sum_i \left(\frac{w_i}{\rho_i}\right) \quad (1)$$

Here ρ_i is the apparent density in solution of component i, and w_i is the mass fraction of component i. The apparent density ρ_i is a function of the solids content in the solution. This equation normally uses the assumption that the volume occupied by each component is not dependant on the presence of other components. There are equivalent equations like Equation (8) using molar volume. Though most of the literature data uses molar basis using mole fractions and molar volumes and while a molar works well for simple well-defined solutions, a mass basis is easier to evaluation for poorly defined solutions such as honey and milk.

McCarthy and Singh (2009) confirmed the use of such an equation to calculate the density of milks and creams. The differences are clearly evident when the available data are converted to apparent densities in solution. For instance, the density data from Chenlo et al. (2002) when converted to the apparent density of the sugar in solution, showed large uncertainties and hence might not be useful for further analysis.

McDonald and Turcotte (1948) measured the densities of lactose monohydrate solution up to a mass fraction of 15% and also fitted old data for supersaturated solutions with concentrations up to 50% at 20 °C and 25 °C. They fitted Equation (2) to their data at 20 ºC.

$$\rho_{lactose}^{20} = 998.2 + 3.70\, w_s + 0.015\, w_s^2 \tag{2}$$

The form of Equation (2) is quite different from Equation (1), reflecting an empirical approach. Temperature dependence ($10, 20, 25$ & 30 °C) of densities for invert sugar solutions were reported by Snyder and Hattenburg (1963). Equations (2-5) were fitted from the measured values of densities at each temperature up to 50% mass fraction solids. Though these equations are empirical and are specific to temperatures, consolidated and generalized thermodynamic equation which should cover the entire composition and temperature range. The application of these equations are limited to the type of solute that is used. In contrast, the current research aims to obtain generalized relationships.

$$\rho_{invert}^{15} = 999.1266 + 3.895362\, w_s + 1.24621e^{-2}\, w_s^2 + 4.632e^{-5}\, w_s^3 - 2.1854e^{-7} w_s^4 \tag{3}$$

$$\rho_{invert}^{20} = 998.2343 + 3.863641\, w_s + 1.214466e^{-2}\, w_s^2 + 5.417e^{-5}\, w_s^3 - 2.5882e^{-7} w_s^4 \tag{4}$$

$$\rho_{invert}^{25} = 997.0770 + 3.827780\, w_s + 1.261903e^{-2}\, w_s^2 + 4.235e^{-5}\, w_s^3 - 1.661e^{-7} w_s^4 \tag{5}$$

$$\rho_{invert}^{30} = 995.6780 + 3.798374\, w_s + 1.264516e^{-2}\, w_s^2 + 4.180e^{-5}\, w_s^3 - 1.5334e^{-7} w_s^4 \tag{6}$$

The density equations where further enhanced to a 9-constant empirical Equation (7) between the temperature range of $0 - 30$ °C and concentration range up to 75% sucrose solution by Barber (1965). Further, a theoretical Equation (8) (kg/m^3) was proposed by Barber (1965) for the temperature range $30 - 60$ °C for up to 70% sucrose solution.

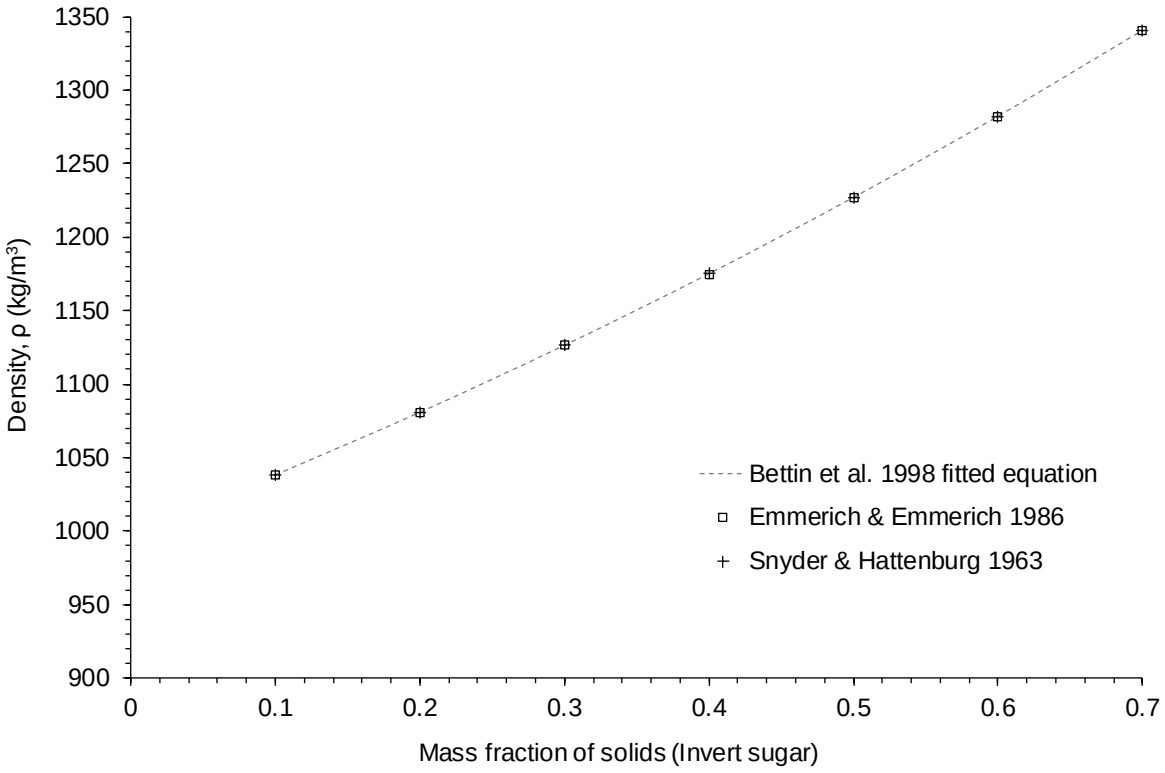
$$\begin{aligned} \rho_{sucrose}^{T} = {} & (1000.3698 + 3.9680504e^{-2}\, T - 5.8513271e^{-3}\, T^2) \\ & + (389.82371 - 1.0578919\, T + 1.2392833e^{-2}\, T^2)\, w_s \\ & + (170.97594 + 0.47530081\, T - 8.9239737e^{-3}\, T^2)\, w_s^2 \end{aligned} \tag{7}$$

$$\rho^{T}_{sucrose} = \frac{x_s M_s + (1 - x_s) M_w}{x_s \bar{V}_s + (1 - x_s) \bar{V}_w} \quad (8)$$

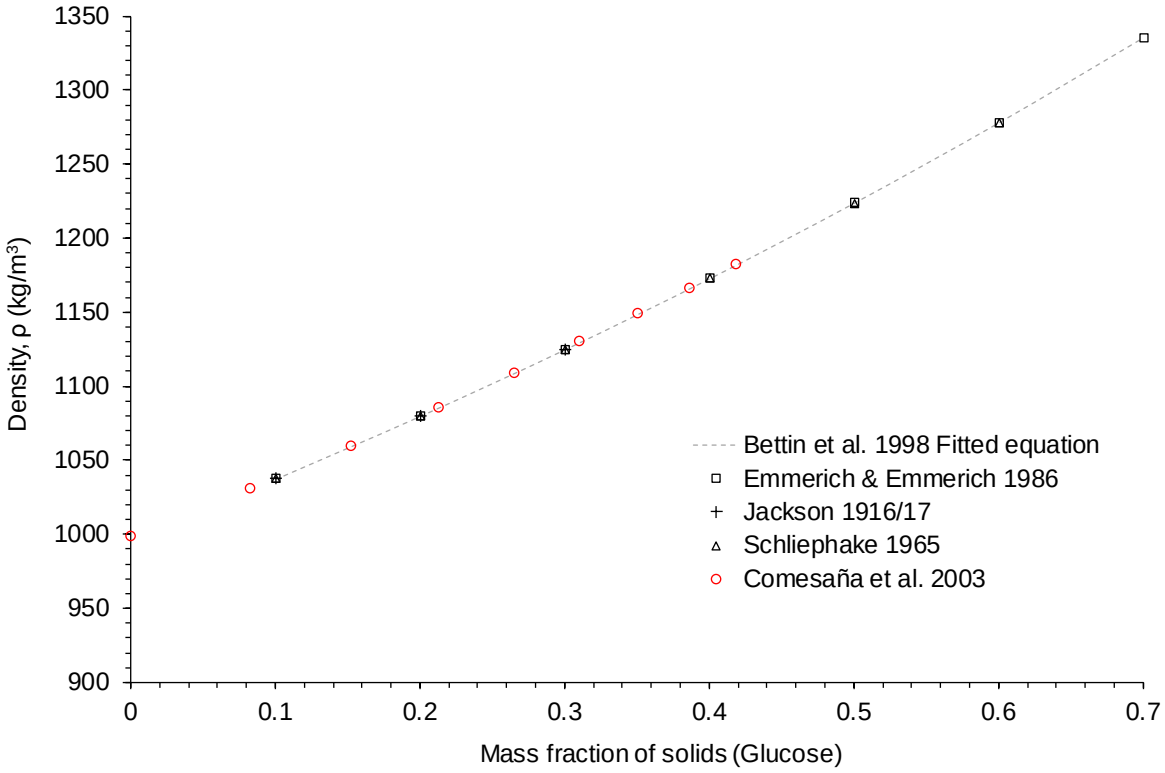
With, $\bar{V}_s = 0.21257059 + 1.3371672e^{-4} - 2.9276449e^{-1}\, T^2$, $\bar{V}_w = 0.018027525 + 4.8318329e^{-1}T + 7.7830857e^{-5}T^2$. Here, x_s is mole fraction, T is in ºC, $\bar{V}_w$ is the molar volume of water (m^3/mol), $\bar{V}_s$ is the apparent molar volume of sucrose in solution and M is the molecular mass in kg/mol.

In 1995, Vázquez et al. (1995) measured densities and viscosities of sodium sulphite + sucrose + water solutions and proposed an equation for density with concentration as a dependent factor. The temperature range for this empirical equation to hold is between $25 - 40$ °C and fairly dilute solutions.

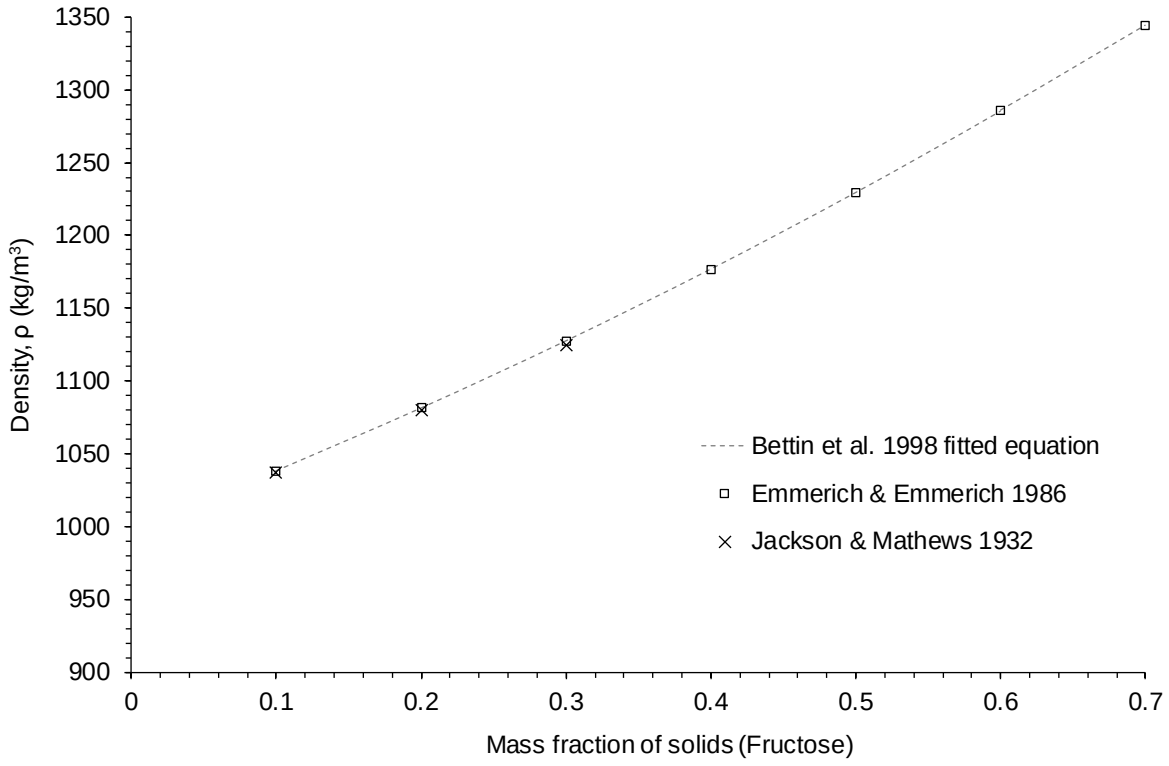
Bettin et al. (1998) tried to develop polynomial density equations based on the mass fraction of the solids and the temperature. They developed polynomial equations that had numerous coefficients and the fitted values were very close to the values reported by Snyder and Hattenburg (1963) and other authors. Hence, summarized in their table was the conformity of the closeness of the data fit. Figure 6 shows the availability of density data and the closeness to the fit of most data set in the literature. This emphasises the point that no further investigation on density data is required.



(a) Invert sugar



(b) Glucose



(c) Fructose

Figure 6 Density data and fits available in the literature for simple sugars. (a) Invert sugar; (b) Glucose; (c) Fructose (Bettin et al., 1998).

A density study conducted on mono-, di-, and trisaccharides + NaCl by Banipal et al. (2002) showed the dependencies of NaCl on the density for monosaccharides like glucose, mannose, and galactose and so on at 25 ºC. Figure 7 shows the density variations of glucose with various NaCl mass fractions. Lactose and maltose were also analysed (not shown) to understand similar trends; a non-linear response was obtained and this is attributed to enhancement in structural order due to the presence of NaCl. The study was not particularly useful as the concentrations of sugar in the systems studied were lower than the complex sugar systems used in the current work.

Excess molar volumes were calculated by Darros-Barbosa et al. (2003). These excess molar volumes were positive for aqueous sugar solutions and negative for salt solutions. Increases in concentration and temperature were shown to increase the deviation of the excess molar volume. Based on the study it was concluded that sugars are 'structure forming' and salts as 'structure breaking'. Darros-Barbosa et al. (2003) used similar equations to Barber's equation, but in terms of mole fractions. The experimental and calculated densities of the model food systems showed considerable variation for a combination of equal concentrations of sugars

(10 % glucose + 10% fructose + 10% sucrose) only. This may be due to the use of mole fractions instead of mass fraction for sugars.

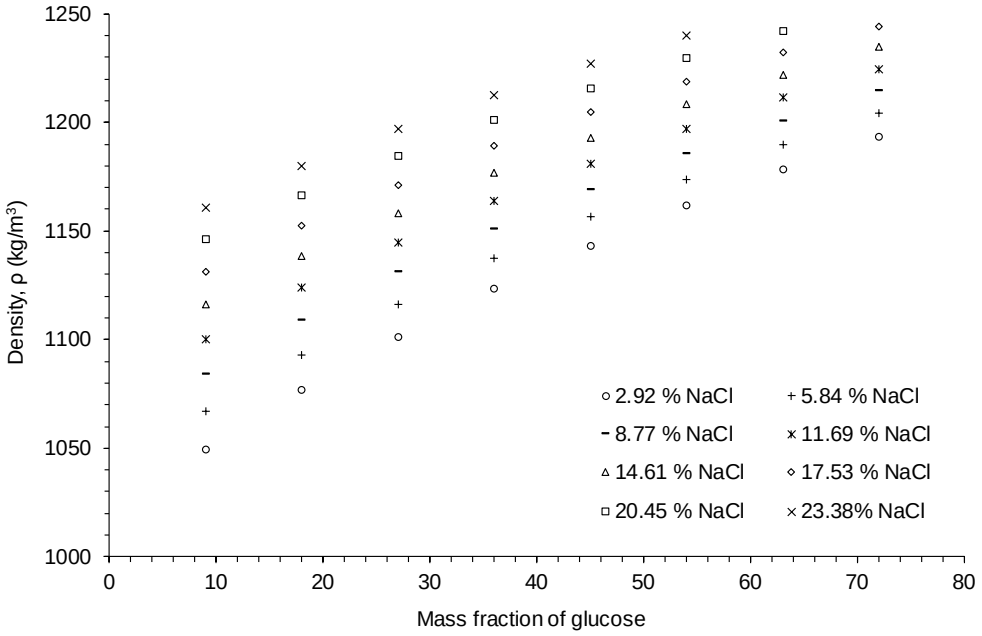


Figure 7 Density of glucose + NaCl + water solution variation with concentration of NaCl at 20 °C (Comesaña et al., 2003)

Fucaloro et al. (2007) extended the work done by Banipal et al. (2002) and developed an equation for the molar volume of sugars at infinite dilution in relation to temperature. Gharsallaoui et al., (2008) interpreted hydration of sugar molecules in solution from the bulk density data in the literature of binary solutions and compared this with hydration numbers from water activity data. Thermodynamic and molecular modelling techniques provided the basic understanding and assumption that with increasing sugar concentration, there was no long range structuring effect of solute on water. Also, increase in temperature and concentration for sugars showed a decrease in the hydration numbers for the sugars. The hydration numbers were in accordance with the Starzak and Mathlouthi (2006) method of calculating hydration number from the activity coefficient data.

The emphasis of the current work is to understand the different empirical and semi-empirical equations and to eliminate them based on statistics. The influence of the parameters that the equations are based on such as temperature, composition, concentration are tested and reported. Equations (2-7) must be classed as empirical equations and may be chosen or avoided in engineering applications as this does not cover the entire temperature and concentration range.

Hence, a more fundamental approach and equations similar to Equation 1 will be applied throughout this work as this is based on the theoretical understanding of density.

2.3.2. Electrical Conductivity

Literature available on the electrical conductivity of honey and sugars systems showed limited data. The measured electrical conductivity of these systems were predominantly correlated to the ash or salt content in the system due to the purity of sugars (Accorti et al., 1987; Kropf et al., 2008). The electrical conductivity (referred to as conductivity below) of honey is limited by the Codex (2001) standard for honey which states that the conductivity should be no more than 0.8 mS/cm. However this conductivity is measured after dilution of 20 g of honey with 100 mL of water so is not directly related to this work. Due to the fact that honey is extremely variable, it is difficult to predict many of its physical properties or relate these to composition.

Similar to other properties of honey, the conductivity varies depending on the geographical and botanical conditions. The conductivity can be used to distinguish whether honey is from honeydew or floral in nature (Ahmed et al., 2007; Belay et al., 2013; Gómez-Díaz et al., 2012). Similarly, Guo et al. (2011) showed that the water contents of honey and honey solutions can also be measured using electrical conductivity measurements. Increases in either water content or temperature resulted in an increase in the electrical conductivity of honey. An empirical quadratic expression for the electrical conductivity of honey was obtained based on water content, and temperature. Apart from helping to distinguish the type of honey, an effort was made to correlate the composition of carbohydrate in honey to the electrical conductivity (Kaskoniene et al., 2010). This study did not yield any relation between the carbohydrate composition and electrical conductivity.

Stokes and Weeks (1964) discuss the interdependence of viscosity effects on electrical conductance of the sucrose polymer Ficoll. The viscosity and conductance of Ficoll solution prepared in weight basis with small quantities of electrolytes with smaller ions like K^+, Cl^-, Na^+, Cl^- and larger ions like $Ca^{2+}, N(C_4H_9)^+$ (tetra ethyl ammonium ion) were measured. The conclusion from the data was that the non-electrolytes in the solution form a network like structure where it is easier for the smaller ions to permeate through as explained by the Stokes' law or the Walden rule. The work in this thesis involves these smaller ions.

Electrical conductivity of an aqueous solution is the manifestation of the movement of ions. The ions of water (H^+& OH^-) are able to move by proton hopping from one water molecule to another, with direct movement of a particular ion. Other ions need to diffuse under the influence of the applied electrical field and hence the solution viscosity is very influential. Ion mobility can be related to diffusion and hence to viscosity, η using the Walden equation.

$$u_i = \frac{e_0 z_i}{6\pi\eta r_i} \tag{9}$$

Here e_0 is the charge of an electron and r_i is the Stokes' radius. However it has been found that as the viscosity increases this behaviour is not followed but instead it has been found that $\lambda \propto 1/\eta^{\alpha}$ where the constant α has been determined to be about 0.78 for monosaccharides (Miller et al., 2000). Therefore, Equation (9) was implemented as Equation (10) which is also known as the fractional Walden Equation.

$$u_i = \frac{e_0 z_i}{6\pi\eta^{\alpha} r_i} \tag{10}$$

A number of researchers have studied saccharide + electrolyte systems and proposed modifications to Walden's rule. Stokes (1959) measured the conductivity of concentrated solutions of sucrose + potassium chloride. He concluded that the limiting conductivity of potassium chloride was inversely proportional to viscosity raised to the power of 0.7 i.e. $\alpha = 0.7$. Miller et al. (2000) found that α was 0.64 for trehalose + sodium chloride and 0.78 for sodium chloride in glucose. These results suggested a correlation between the exponent and the size of the sugar. Longinotti and Corti (2009) measured conductivity of various electrolytes in sucrose and trehalose and found that α ranged from 0.61 to 0.74 and depended on the size of the electrolyte ions and the sugars.

Conductivity measurements on highly concentrated maltose + water + KCl mixture shows that the mobility of ions have a decoupling effect on viscosity; and conductivity measurements has become a useful tool in understanding these concentrated systems (Noel et al., 2000). Longinotti and Corti (2004) studied the ion mobility of smaller ions in sugar solutions and compared with that of larger ions like borate with sucrose and trehalose. While the smaller ions obey the Walden rule, the larger ions were found to have differences and deviated from the Walden rule as shown in Figure 8 and a modified or fractional Walden product (Equation 11)

seemed to be a clear indicator of the trend. This was attributed to the formation of the smaller esters by trehalose with the borate ion compared to sucrose-borate esters.

$$\Lambda\eta^{\alpha} = constant \tag{11}$$

Though this work is beyond the scope of this project, this work can be used as a confirmation of the Walden rule on a model food system like model honey. In a similar type of work, Longinotti et al. (2002) concluded "*It is believed that the reason for the enhanced ion mobility in the aqueous sugar solutions is the existence of structural microheterogeneities in the fluid. The local environment around the ions would be richer in water and consequently the local viscosity will be lower than the bulk viscosity.*"

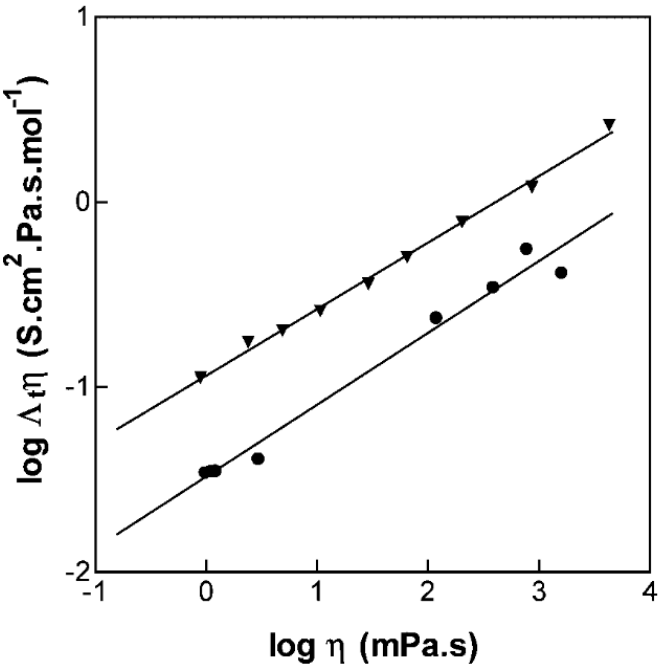


Figure 8 Walden product as a function of viscosity in trehalose aqueous solutions at 25 ºC sodium borate (●) and NaCl (▼) (Longinotti & Corti, 2004).

Hu et al. (2010) favoured a form with $\eta^{0.5}$ which they used for binary solutions of a component with water activity that is same as the water activity of the mixed solution. This approach, while valid, is problematic at very high sugar concentrations where water activity is not well defined for all solutes.

Diffusion is another parameter that needs to be understood in these mixtures. The Nernst-Einstein Equation (Equation 12) explains the relation between diffusion and electrical

conductivity (Atkins, 1978; Robinson & Stokes, 1965) where Λ_m is the molar conductivity, z is the ionic charge, F is Faraday's constant, R is universal gas constant and T is the temperature.

$$\Lambda_m = \frac{z^2 F^2 D_i}{\mathrm{RT}} \tag{12}$$

The diffusion counterpart to the Walden Equation (Equation 9) is the Stokes-Einstein relation (Equation 13) where D is the diffusion coefficient and k_B is Boltzmann's constant and T is the temperature. Hence, conductivity measurements can be used to understand the diffusion mechanisms and characteristics of ions in these sugar mixtures.

$$D = \frac{k_B T}{6\pi\eta r_i} \tag{13}$$

Miller et al. (2000) discussed the effective use of a Walden type relation similar to Equation 11 in order to get the diffusional equivalent of conductivity and this can be simplified as Equation (15)

$$D = \frac{k_B T}{6\pi\eta^{\alpha} r_i} \tag{14}$$

$$D\eta^{\alpha} = constant \tag{15}$$

Many liquid foods such as milk, yogurt, and honey are complex solutions and colloidal dispersions containing sugars, proteins, minerals, fat and other minor components in water. Knowledge of property contributions by each component in milk will provide a clear understanding of the liquids characteristics (Madoumier et al., 2015).

Sharifi and Young (2012) established a correlation between conductivity and the composition of concentrated milk solutions with up to 47% solids and further suggested the use of conductivity as an online measurement tool to correlate to milk concentration (Sharifi & Young, 2013). They used multiple linear regression to relate total solids content to temperature and electrical conductivity, but they did not consider the effect of viscosity. Another factor that need to be considered here is that experimental data used in these regression was for milk solutions up to 30% by weight while much higher concentrations could be achieved.

St. Gelais and Champagne (1995) measured conductivity and viscosity of milk solutions as the pH was reduced. While their data show that both measurements changed, the changes appeared

to be independent of each other. When the pH changed rapidly from 5.6 to 5.0, the viscosity increased about 100 times, while the conductivity only doubled. Henningsson et al. (2005) stated that proteins and lactose affected electrical conductivity of milk via viscosity, and that proteins, being charged, could contribute directly as a charge carrier.

Bordi and Cametti (1986) showed that the concentration (and hence the bulk viscosity) of sodium carboxymethyl cellulose (NaCMC) has little effect on electrical conductivity when the concentration of $BaCl_2$ was greater than about 3 mmol/L. Below that concentration they used Manning ion condensation concepts to explain the effect of NaCMC concentration. They also noted that aqueous solutions of sodium polymethacrylate and polystyrenesulfonate, a lower molecular weight polymer relative to CMC, was also associated with higher conductivities.

2.3.3. Viscosity

Viscosity of sugar solutions provides information on the transport properties. Viscosity data can be used to understand the molecular association, and hydration of sugars when interpreted with properties like water activity (Starzak et al., 2000). The understanding of binary sucrose solutions can be extended to complex sugar mixtures like honey.

Yanniotis et al. (2006) confirmed that decreasing the moisture content of a honey solution will result in an increase in the viscosity. This result was determined when measurements of moisture content were carried out on several samples of honey, each pertaining to a different botanical origin. In addition to this, previous studies into the effect of altering the sugar concentration in binary mixtures of water and sugar have been performed and it has been found that increasing the concentration of sugar in solution increases the viscosity (de Souza et al., 2010; Oroian, 2013; Telis et al., 2007). In the experiment performed by Telis et al. (2007) it was observed that the viscosity of sucrose was the greatest followed by glucose then fructose at the same concentration. Results for glucose were not obtained at concentrations greater than 40% by mass.

Magazu et al. (1998) used the model proposed by Mooney and Padova with volume fraction as the basis for viscosity and discussed diffusion characteristics in saturated and supersaturated trehalose systems. Equation 15 was applied to the understanding of these concentrated sucrose

solutions without the correction factor α and hence there was deviations. Bakier (2007) measured the effect of water content on the viscosities of various honeys empirically and stated the results in the form of an equation which makes it easier to calculate the viscosities using measured water content. Though this is a scientifically acceptable practice, this empirical equation is based on a particular origin and cannot be generalized for all honeys.

Monosaccharides with axial and equatorial orientation of hydroxyl groups at the first (C1) and fourth (C4) carbon atom in pyranose ring decrease the viscosity more than monosaccharides with two axial hydroxyl groups in these positions. Due to the differences in the molecular structures of saccharides resulting in different shapes of molecules and different compatibility levels to the water structure, the saccharides differ in ability to disturb water layers around alumina particles (Falkowski & Szafran, 2016).

Little data is available for viscosity of sugar solutions with more than two components in the literature. However, measurements of the viscosity of ternary solutions containing water, sodium chloride and a sugar have been carried out at varying temperatures (Comesaña et al., 2003). The viscosities of ternary mixtures of sugars solutions at various concentrations were measured and data were presented as shown in Figure 10 (Banipal et al., 2006; Moreira et al., 2003; Roos, 2009). Though, these viscosity data are useful, the concentration of solutions measured is for a dilute solution. Flood and Puagsa (2000) reported the viscosity and solubility of sugars in solutions of fructose + glucose + ethanol + water with various ethanol: water ratios.

The rheological properties of honeys have been studied based on the composition, botanical origin and temperature in the literature (Bhandariet al., 1999; Gomez-Diaz et al., 2009; Lazaridou et al., 2004; Mossel et al., 2003; Recondo et al., 2006; Zaitoun et al., 2001). However, this work focusses on quantifying the contribution of various sugars present in complex sugar mixtures.

Bhattacharyya and Bagchi (1997) predicted the transportation properties like diffusion of small solute and water molecules in dense solutions using first principles. Similar to Miller et al. (2000)’s work on understanding the electrical conductivity of concentrated aqueous sugar solutions, Rampp et al. (2000) measured the temperature, concentration dependence of

viscosities. Further, Rampp et al. (2000) measured and summarised the diffusion co-efficient using the Stokes-Einstein equation for sugar solutions at various concentration.

Considerable contributions towards the understanding of the transport properties like diffusion and viscosity of concentrated sugar solutions have been studied by the group over a period of time (Corti et al., 2008; Longinotti & Corti, 2004; Longinotti et al., 2002; Miller et al., 2000; Miller et al., 1999). The effects of salts, smaller molecules on the viscosity and electrical conductivity of concentrated sugar solutions were studied by Miller et al. (2000). The understanding of the effect of viscosity on the self-diffusion of smaller sized particles and methods to decouple their effects has been a major contribution by this group.

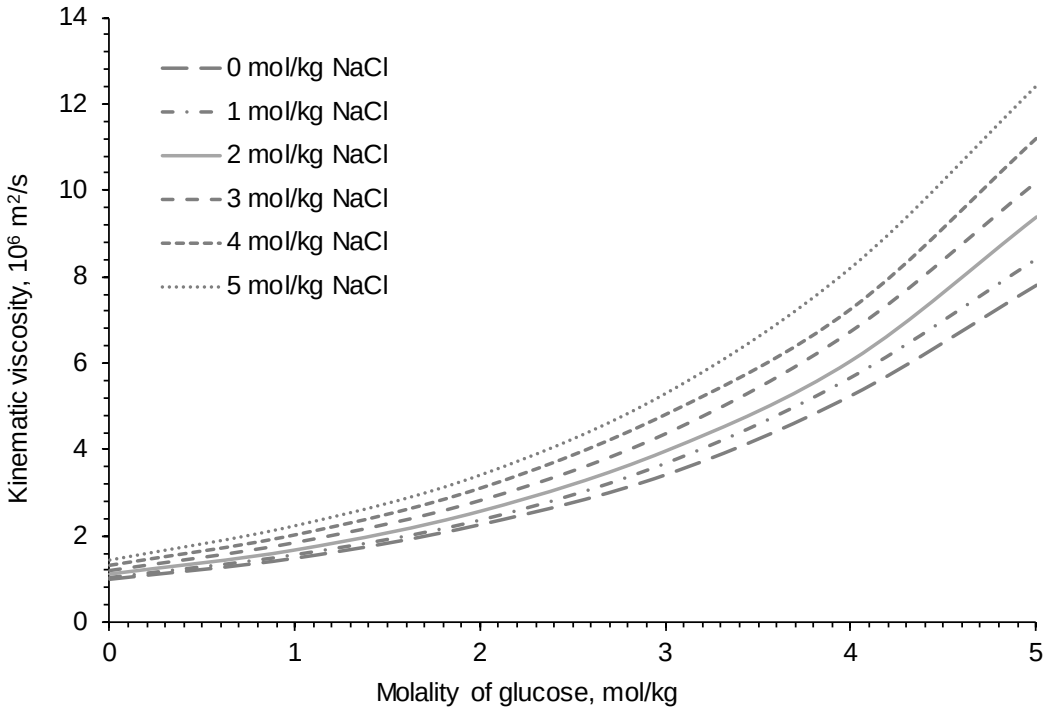


Figure 9 Kinematic viscosities of ternary solution of glucose + NaCl + water at 20 ºC (Moreira et al., 2003).

2.3.3.1. Viscosity Models

The most common models for the effect of temperature on viscosity is the Arrhenius type model. The Arrhenius model though used widely, does not fit a system like honey or any other food systems well (Bhandari et al., 1999). An aqueous sugar solution behaves as a Newtonian fluid and therefore the viscosity is expected to decrease with increasing temperature. This phenomenon can be described using the Arrhenius model as reported by Telis et al. (2007).

However, the focus is on varying the sugar composition at isothermal conditions. As will be discussed in the results section, exponential type equation will be used with contribution from water and the sugar in the system accounted for.

Williams, Landel and Ferry (WLF) Model: The WLF equation incorporates the concept of glass transition temperature and relates viscosity for a number of polymers. T_g is the glass transition temperature and η_g is the viscosity at glass transition of the polymer while C_1 and C_2 are constants. This approach is to understand the effect of temperature on the viscosity of the solutions.

$$\log\left(\frac{\eta}{\eta_g}\right) = -\frac{C_1(T - T_g)}{C_2 + (T - T_g)} \tag{16}$$

Vogel-Taumman-Fulcher (VTF) Model: The VTF model is similar to the WLF model with the difference is at using the viscosity at $T = \infty$ instead of glass transition.

$$\frac{\eta}{\eta_\infty} = exp\left[\frac{B}{T - T_0}\right] \tag{17}$$

T_0 is the temperature where the molecular displacements are infinite and B is a constant

Power law model: Hill and Dissado (1982) proposed the power law model

$$\eta = k(T - T_g)^{-m} \tag{18}$$

Here, k and m are constants. These models explain the dependence of viscosity on temperature. It is summarised by Recondo et al. (2006) that the VTF model helps explain this effect better when any form of extrapolation is required for sugar systems. Equations (16-18) have an important restriction that they only apply at temperatures at or above the glass transition temperature.

Rudgers (1962) did a comprehensive review of over 170 equations that relate viscosity to concentration in terms of mass fraction of water and sugars. Krieger and Dougherty (1959) described effect of concentration on the viscosity of concentrated suspension based on the maximum packing fraction ϕ_m and related the viscosity using the exponent n, as $n = [\eta] \cdot \phi_m$ and $[\eta]$ is the intrinsic viscosity. Equation 19 was the basis for this understanding where, η_r is the relative viscosity.

$$\eta_r = \left(1 - \frac{\phi}{\phi_m}\right)^{-\mathrm{n}} \tag{19}$$

The Mooney equation for non-diluted suspensions is given by Equation (20), where, ϕ is the volume fraction and k is the interaction parameter for the particles. The range of k is reported in the range as 1.35 to 1.91 as this is obtained empirically (de Souza et al., 2010).

$$\eta_r = \exp\left(\frac{5}{2}\left[\frac{\phi}{1 - k\phi}\right]\right) \tag{20}$$

Morison and Hartel (2007) proposed an equation based on mass fraction of the components after carefully reviewing and analysing the set of equations analysed by Rudgers. This equation is similar to the form proposed by Mooney and an empirical extension to the theoretical form where, a_i is a temperature dependent constant for component i, w_i is the mass fraction of component i, and w_w is the mass fraction of water.

$$\ln(\eta_r) = f(w) = \sum_i \frac{a_i w_i}{w_w} \tag{21}$$

When a mixture is considered, the best representation is in mass fraction rather than the volume fraction as a number of factors like hydration water and the competition of available water needs to be factored. Hence, as suggested by Morison et al. (2013), an updated equation with a mass fraction basis will be used in this work.

Madoumier et al. (2015) after testing different models stated that the model proposed by Morison et al. (2013) was satisfactory at modelling milk viscosity in evaporators and other unit operations in the milk concentration process. This model for viscosity is extended to food systems with sugars.

2.3.4. Refractive Index

The refractive indices of lactose solutions were measured by McDonald and Turcotte (1948) up to mass fraction of 36% at 15, 20 and 25 °C and empirical equations determined. Similarly, Snyder and Hattenburg (1963) measured the refractive indices of invert sugar solutions up to 85% mass fraction and developed equations as follows. One of the key observations by Snyder and Hattenburg (1963) was that the refractive indices of the invert sugar solutions were lower

than that of sucrose solutions for the same concentration. Equations (22 to 25) describe the refractive indices at $15, 20, 25$ and 30 °C respectively. Again, most of these empirical equations do not provide a consolidated equation over a range of temperatures and composition. This is precisely why this current research aims to obtain relationships which can provide a common equation.

$$n_{invert}^{15} = 1.3333872 + 1.4292e^{-3}\, w_s + 0.515855e^{-5}\, w_s^2 + 0.1153e^{-7}\, w_s^3 \quad (22)$$

$$n_{invert}^{20} = 1.3329877 + 1.4149e^{-3}\, w_s + 0.52729e^{-5}\, w_s^2 + 0.1105e^{-7}\, w_s^3 \quad (23)$$

$$n_{invert}^{25} = 1.3325026 + 1.4114e^{-3}\, w_s + 0.51088e^{-5}\, w_s^2 + 0.1244e^{-7}\, w_s^3 \quad (24)$$

$$n_{invert}^{30} = 1.3319403 + 1.4016e^{-3}\, w_s + 0.5134e^{-5}\, w_s^2 + 0.1249e^{-7}\, w_s^3 \quad (25)$$

Ruddick and Shirtcliffe (1979) derived empirical equations for sucrose + NaCl solutions at different temperature and total solids concentration within 40% mass fraction. Equation 26 also incorporates the interaction parameters between NaCl and sucrose. However, the limitations still remain in the concentration range and does not deal with solutions of high concentrations.

$$\begin{aligned} n^{20} = 1.3330 &+ 0.014398\, w_{sucrose} + 0.04685\, w_{sucrose}^2 + 0.0254\, w_{sucrose}^3 + 0.1748\, w_{salt} \\ &- 0.0021\, w_{salt}^2 + 0.069\, w_{salt}^3 + 0.0991\, w_{sucrose} w_{salt} \\ &+ 0.1558\, w_{sucrose}^2 w_{salt} + 0.0273\, w_{sucrose} w_{salt}^2 \end{aligned} \quad (26)$$

Refractive index of fructose + glucose + ethanol + water was measured by Flood and Puagsa (2000) at 25 °C as part of a study to understand the crystallization characteristics of a complex system as employed in the chromatographic separation of crystalline fructose. It was concluded that the refractive index is a measure of the total solute in the system and the change in the refractive indices due to changes in solvent concentration also did not have any significant effect at higher sugar concentrations.

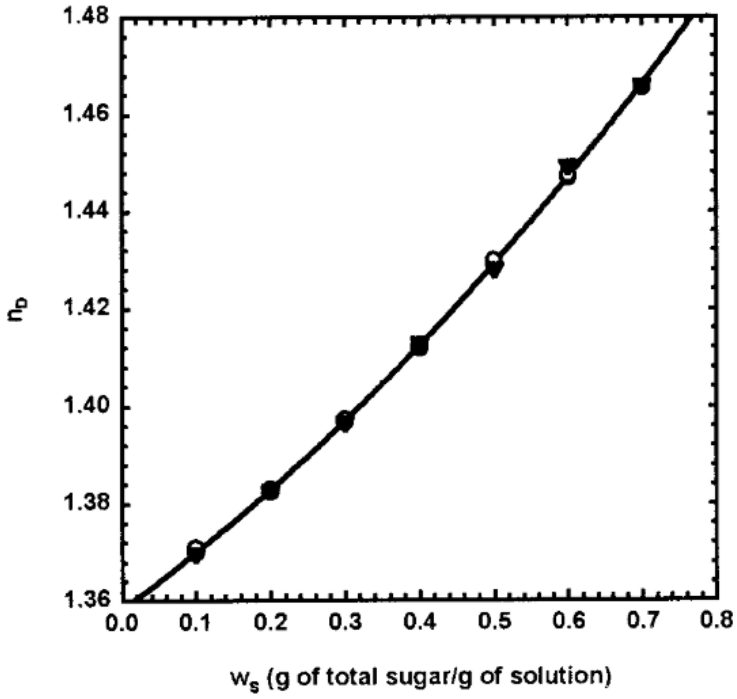
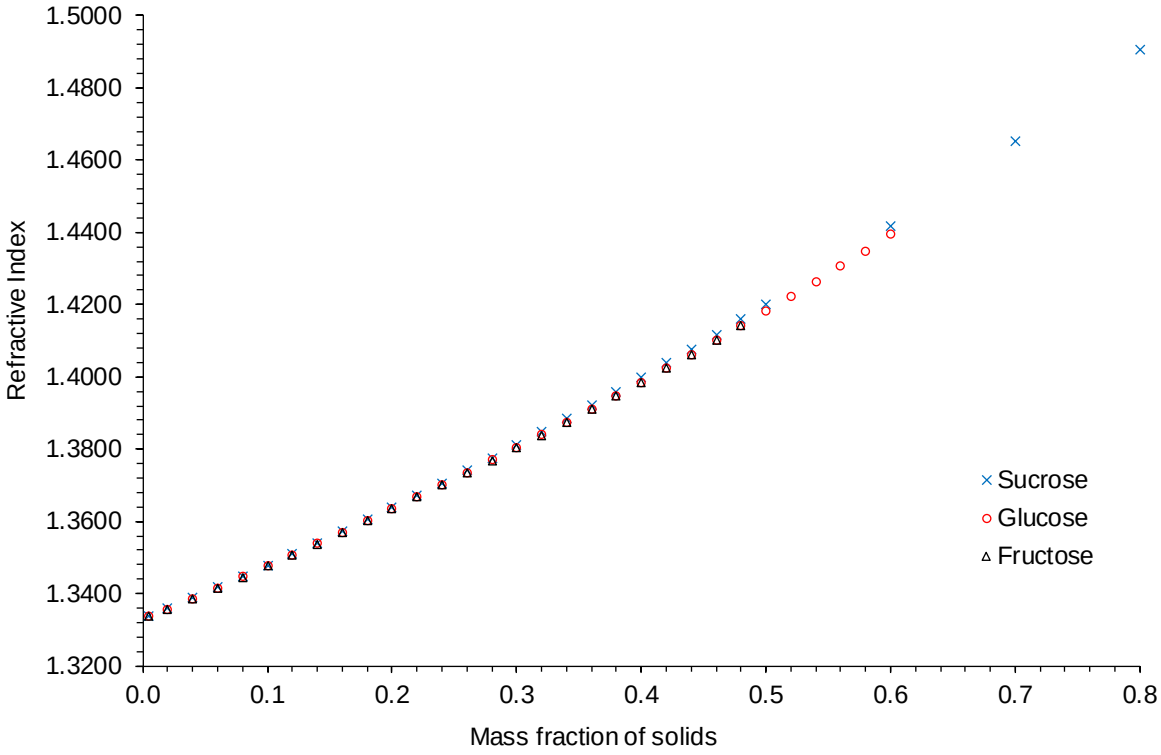
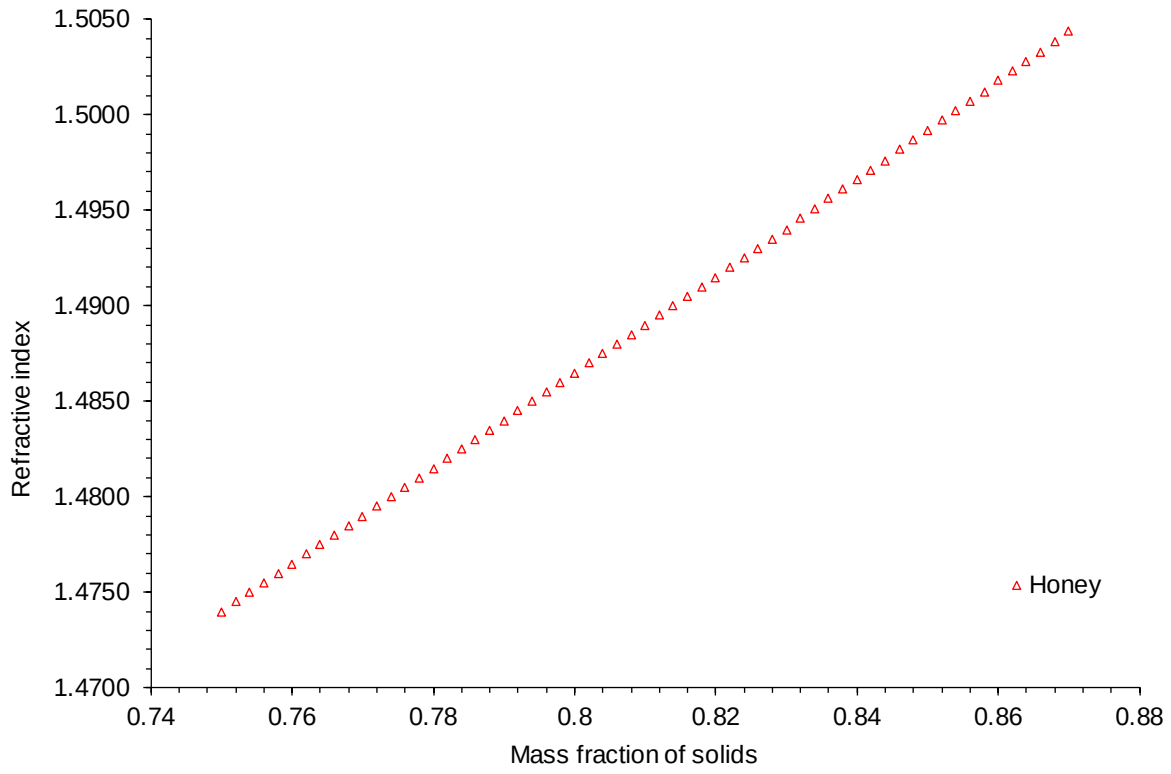


Figure 10 Refractive index for solutions of glucose and fructose in ethanol-water mixtures of 40 mass % ethanol. Ratio of glucose fructose: ●, 1:1; ○, 2:1; ▼, 1:2. (Flood & Puagsa, 2000)



(a)



(b)

Figure 11 Refractive Index available in the literature. (a) The graph on the left shows the refractive index of sucrose glucose and fructose (Weast, 1978). (b) The graph on the right shows the harmonized values of refractive index of honey (Bogdanov, 2009).

As discussed for density, the equations currently available for refractive index are based on empirical values and some defined parameters. Figure 10 and Figure 11 above show the comparison of refractive index of binary solutions of sugar on the left and the refractive index of honey in the right. Visual curvature can be seen on the refractive index of binary solutions while the honey shows absolute linearity and this needs comprehension and hence, a mixing rule will be discussed and evaluated. Additionally, refractive index was used as a tool to confirm the composition of the prepared sugar system. Complex systems with more than three components will also be evaluated and discussed based on the mixing principle.

Reis et al. (2010) proposed a new thermodynamic approach towards determining the refractive indices of binary liquid mixtures as literature regarding this is scarce. Here, the refractive indices of the components of a solutions before mixing were considered. When, considering a binary sugar solution before mixing, we can consider two different layers and hence, the interrelationships between refractive index and molar volume is applied as in Equations (27).

$$n\,(unmixed) = \phi_{sugar} n_{sugar} + \phi_{water} n_{water} \tag{27}$$

where, $\phi_{sugar}, \phi_{water}$ are the volume fractions of sugar and water respectively and n_{sugar}, n_{water} are the refractive index of pure component at the same concentrations. Assuming ideality of the binary solutions, and applying Maxwell's relationship (Equation 28) of permittivity and refractive index Equation (27) can be used as Equation (29).

$$\epsilon = n^2 \tag{28}$$

where, ϵ is the dielectric permittivity and n is the refractive index. Equation (28) was deduced and used by Huang and Sarkar (1978) to calculate the refractive index of silica glass mixtures. Reis et al. (2010) gave a thorough review of equations that might be used for the refractive index of mixtures of two liquids, but several options are given in literature, e.g., Yahya and Saghir (2015). Reis et al. (2010) concluded that the most likely thermodynamic equation form for an ideal mixture was Equation (29). This will be applied here for the mixture of a solute (B) and solvent (A).

$$n^{id} = (\phi_A (n_A^*)^2 + \phi_B (n_B^*)^2)^{0.5} \tag{29}$$

Here the volume fractions can be based on specific (or molar) volume of pure solvent and of the apparent specific (or molar) volume of the solute at infinite dilution. The volume fraction, e.g. ϕ_B, can be calculated from Equation (30).

$$\phi_B = \frac{\frac{w_B}{\rho_B}}{\frac{w_A}{\rho_A} + \frac{w_B}{\rho_B}} \tag{30}$$

Here w_A and w_B are the mass fractions of A and B, ρ_A is the density of pure solvent and ρ_B is the apparent density of solute in solution. Reis et al. (2010) proposed that the deviation from ideality is best defined as the excess squared refractive index

$$(n^2)^E = n^2 - (n^2)^{id} \tag{31}$$

And that the excess be expressed in terms of the Redlich-Kister equation

$$(n^2)^E = \phi_A \phi_B \sum_{k=0}^{m} A_k (2\phi_A - 1)^k \tag{32}$$

for $m = 1$ as was found to be shown to be sufficient

$$(n^2)^E = \phi_A \phi_B (A_0 + A_1 (2\phi_A - 1) \tag{33}$$

Rearranging we get

$$n^2 = (\phi_A(n_A^*)^2 + \phi_B(n_B^*)^2) + \phi_A\phi_B(A_0 + A_1(2\phi_A - 1) \quad (34)$$

When these equations are combined there are three fitting parameters: n_B^*, A_0, and A_1.

2.3.5. Water Activity

Water activity is a prominent measurand in the food industry which helps predict properties like shelf life and microbial activity (Ross, 1975). Water activity of a food system can be understood as the fraction of water that is freely available. Water activity according to Raoult's law is defined as the ratio of the vapour pressure of water, p, above the sample to the vapour pressure of pure water at the same temperature, p_0, and can be expressed in terms of water activity coefficient, γ_w and the mole fraction of water, x_w.

$$a_w = \frac{p}{p_0} = \gamma_w x_w \quad (35)$$

Zamora and Chirife (2006) stated that every compound will have a constant value of water activity when saturated in water. For example, the water activity of saturated glucose in water at 25 ºC is 0.891 (Rüegg & Blanc, 1981) and pure water has a water activity of 1.0 at the same temperature.

2.3.5.1. Water activity measurements on food systems

Teng and Lenzi (1974) fitted the measured water activities of non-electrolyte solutions in the form of a polynomial expression. Lerici et al. (1983) used the freezing point depression as a tool to calculate the water activity of liquid food systems and conclude that this is a very accurate and efficient way to calculate water activity. Comesaña et al. (1999) measured the water activity of sugar + salt + NaCl at 25 ºC and fitted the data to an empirical equation. Mazzobre et al. (2001) studied trehalose and sucrose solutions with salts and their effect on water crystallization on the principle basis of cryopreservation. The conclusion of this study was that the ice crystallization of water reflects the dynamics involved in the water + salt + sugar interactions.

Honey normally has a water activity of less than 0.6, however this can vary in relation to the amount of crystallization in solution. Bakier (2009) showed that the average water activities for liquid honey can range between 0.556 and 0.596, and that of the same types of crystallised

honey are between 0.585 and 0.639 (Gomez-Diaz et al., 2009). Crystallised honey has a higher water activity than in liquid form Bakier (2009), making it susceptible to fermentation.

A fresh food polymer science approach was incorporated in the understanding of water activity in food system, stability over a wide range of temperatures and concentrations (Slade & Levine, 1991), however, this approach will not be used in this work as a more fundamental approach based on physiochemical properties will be used. Glass transition temperature was the rationale behind this polymer science approach.

2.3.5.2. Water activity and microbial growth

The thermodynamic activity of water, water content and water structure in solutions needs to be understood to approach food storage stability and shelf-life (Blandamer et al., 2005; Mathlouthi, 2001; Sablani et al., 2007). The water activity (a_w) of honey directly influences the stability of the product by preventing or limiting microbial growth (Mathlouthi, 2001; M. S. Rahman, 2009; Sablani et al., 2007). Yeasts able to tolerant high sugar concentrations are present in honey and activity of these yeasts in the presence of glucose and fructose results in fermentation. During this time, carbon dioxide and ethyl alcohol are formed, changing honey quality, taste, colour and decreasing shelf life. The alcohol produced from fermentation can then be further broken down making honey taste sour (Chirife et al., 2006). It is known that most moulds however, require a water activity of at least 0.75 to grow. At water activities below this, the stability of the honey is at an acceptable level due to the low microbial activity (Beuchat, 1983; Chirife et al., 2006).

2.3.5.3. Water interactions in food systems

There are three types of interactions that occur in aqueous sugar solutions and include, sugar-sugar, sugar-water and water-water interactions (Starzak et al., 2000). These interactions have been reported to be responsible for most of the biological features in the aqueous solutions and range from gel formation, sweet taste, induction, storage, to stabilization and adherence. Gaida et al. (2006) reported that sugar interactions with a solvent are also responsible for

physicochemical properties such as glass transition temperature, melting temperature, freezing point, solubility and water activity.

Some of the physical properties like the hydration of sugars and activity coefficients at very high concentrations of sugar in solution can be implied from the solute-water interactions. The hydration of sucrose in an aqueous solution has been extensively researched in the past (Starzak et al., 2000). It was reported that although sucrose and water have the same active groups which are involved in establishing hydrogen bonds, the interactions between the molecules are very complex, and the hydration of the sugar is specific for the conformation and configuration of the sugar (Lemieux & Pavia, 1969). It has been found that with increasing concentration of sucrose in solution, additional conformations of the molecule are present which affect the types of bonds formed and as a result, the amount of hydration which can occur (Starzak & Mathlouthi, 2002).

2.3.5.4 Water activity and crystallization

During crystallization, the amount of free water in the solution, water activity is increased as the formation of glucose monohydrate releases two water molecules to be free in solution. Crystallization also removes glucose from the liquid phase as a solute, further decreasing the solids content in the bulk solution indirectly increasing water activity and allowing the naturally occurring osmophillic yeast cells in honey to multiply (Doner, 1977). Zamora and Chirife (2006) gave data showing that water content and water activity have a linear increasing relationship in liquid honey. However, when a saturated solution becomes supersaturated by heating, such as a mixture of crystallised honey to re-dissolve the glucose crystals, the water activity of the solution will decrease (Zamora & Chirife, 2006). Bhandari and Burel (2007) proposed empirical equations to measure the crystal content using the measured water activity.

Rüegg and Blanc (1981) investigated the change of water activity due to crystallization by measuring the water activity of numerous honeys in the liquid and crystallized forms and compared the results. The outcome was an average water activity increase of 0.032 with a standard deviation of 0.028 (Figure 12) and this can be used as a potential tool to determine the onset of crystallization in honey during storage and processing. Gleiter et al. (2006) found and confirmed that the water activity of crystallised honey is higher than liquid honey and also

stated that the main contributor in the crystallization process is glucose and stated that the glucose/fructose ratio aids in microbial growth. As shown there is no observable correlation between water content and decreasing the water activity value. They suggested the lack of an observable correlation was due to the different extent of crystallization in the multiple samples.

Price et al. (2014) measured heavy water (D_2O) diffusion in high viscosity sucrose solutions and calculated the water activity based on the measure relative humidity of the local atmosphere. The results of diffusion coefficient and water activity were linearly fitted empirically using a third order polynomial. This establishes that a relationship between water activity and diffusion does exists in high viscous sugar systems. Further, this work investigated any relationship between water activity and viscosity.

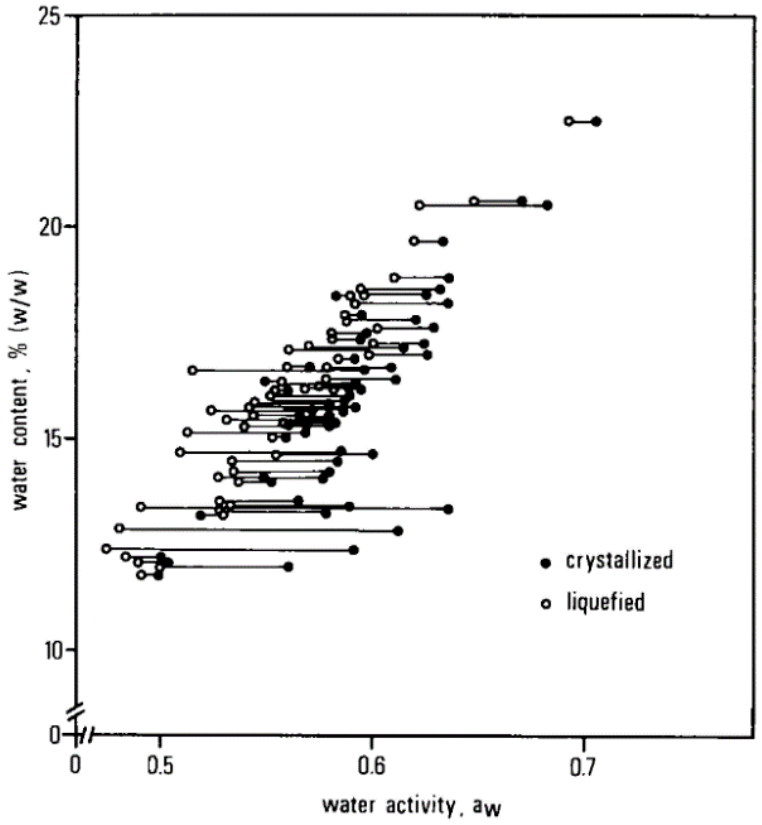


Figure 12 Relationship between water content and water activity (a_w)of crystallised and liquefied honeys. The bars connect the(a_w) values of the same sample before and after liquefaction at 42 ± 2 °C; values were measured at 25 °C (Rüegg & Blanc, 1981) .

Models for water activity predictions are as follows as summarised from Rahman (1995); Barbosa-Cánovas and Vega-Mercado (1996) and Fontana Jr. (2007).

2.3.5.5. Water Activity Models for non-ideal solution

The Grover model (1947) is an empirical equation (Equation 36) that was originally introduced to estimate the water activity of candy formulation and assumes a sucrose equivalent of each component in the candy

$$a_w = 1.04 - 0.10\,p\, + 0.0045\,p \tag{36}$$

where, $p\ =\ \delta_i/W_i$ and W_i is kg water/kg solute and $\delta_i$is a constant for different solutes. Such an empirical equation such as this is clearly wrong because it fails for an infinitely dilute solution.

Norrish (1966) proposed the first simple exponential equation for correlating water activity data in non-electrolyte solutions which may be written in the form,

$$a_w = x_w\left[e^{k\,x_s^2}\right] \tag{37}$$

where, x_w, x_s are the mole fraction of water and solute and k is the empirical constant (sometimes also represented in equations with a negative symbol as the value of k is usually negative) for the solute. Equation (37) is the most fundamental equation from which most models are developed. However, there is still apprehensions about the use of this equation for concentrated solutions due to the large errors in calculated data. Bhandari and Bareyre (2003) showed that the Norrish equation could not predict the water activity value of sugar solutions in the mid-level of supersaturation.

The Teng & Lenzi (1974) model is an arbitrarily fitted polynomial equation which has the form of Equation (38)

$$a_w = 1 + \sum_{i=1}^{k} A_i m^i \tag{38}$$

where, m^i is the molality of component i and the optimum fit is obtained by increasing the value of k. In most cases k is ≤ 10.

Caurie (1985) derived his equation from the Gibbs-Duhem equation and for a three-component system; the water activity can be calculated with the following Equation (39) which is based on three components (n).

$$a_w = a_1 a_2 a_3 - \left[\left(\frac{n(m_1 m_2 + m_1 m_3 + m_2 m_3)}{55.5^2}\right) + \frac{(n+1) m_1 m_2 m_3}{55.5^3}\right] \quad (39)$$

where, m_1, m_2, m_3 are the molalities of the three solutes. The number of components can be increased and the equation can be modified accordingly.

The Lilley & Sutton model (1991) was based on size of solute and solvation. The originally proposed equation was simplified for a ternary solute system that was similar to Ross equation (below) and also concluded that the prediction of water activities by Equation (40) is significantly better than Ross (1975) model.

$$a_w = \frac{a_{12} a_{13} a_{23}}{[a_1 a_2 a_3]} \quad (40)$$

Equations of this form become very difficult to evaluate when there are more than three components because they include all the possible binary combinations.

To summarise, most of these empirical and semi-empirical models fail at extremely high concentration of sugar solutions and is not applicable for most temperatures. Each model has its own drawbacks as specified then and there. Hence, the need to develop better model for water activity data and this work aims at doing so.

2.3.5.6. Models for multicomponent mixtures (ternary or complex)

The Ross model (1975) is an alternative approach equation which gives the water activity of a mixture as the product of the component water activities.

$$a_w = \Pi\, a_i \quad (41)$$

where, a_i is the water activity of individual components in binary solutions at the same molality as the mixture. Ross's equation does not consider the competition for hydration water at low water concentrations. Though the Ross equation is used to test different food systems, the disadvantage is that the water activity of individual components must be measured to evaluate

the overall water activity. Chen (1990) expressed his reservations of Ross equation predicting water activities of solutions containing NaCl, $CaCl_2$ due to the discrepancies he found while calculating using such an equation.

Ferro-Chirife-Boquet (1981) suggested a model that can be used for multicomponent mixtures and is given by the Equations (42-44) (Fontan et al., 1981). The fundamental approach from the Norrish equation is used in this model.

$$a_w = x_w\left[e^{k' x_s^2}\right] \tag{42}$$

$$k' = \sum k \cdot w_s \left[\frac{M_t}{M_s}\right] \tag{43}$$

$$M_t = \sqrt{\sum\left(\frac{w_s}{M_s}\right)} \tag{44}$$

where, x_s, x_w are the mole fractions of the solute and water, k is the Norrish k values from the Norrish equation, w_s is the mass ratio of the solute to the total solids in the mixture, M_s is the molecular weight of each component. We have observed that while testing the use of this equation, it is found that dimensional inconsistencies exists and hence cannot be used. The Lang-Steinberg equation (Lang & Steinberg, 1981) model of multicomponent systems, showed consistent errors in measured and predicted values throughout the range and hence this was discarded.

2.3.5.7. Group contribution models

There are numerous group contribution models which incorporate different interactions like solute-solvent, solute-solute, solvent-solvent and inter-component interactions. UNIQUAC (Peres & Macedo, 1997; Velezmoro et al., 2000), UNIFAC (Marcolli & Peter, 2005), NRTL (Filho et al., 2002), and ASOG (Ninni et al., 2000; Sereno et al., 2001) are some of the group contribution models that are used in the literature to predict properties of sugar solutions. This work does not enumerate these models as an emphasis is made to understand the properties with proposed models for individual properties.

2.3.5.8. Water Activity coefficient

Miyawaki (1996) calculated the water activity coefficient of water from the freezing point depression by using Hildebrand and Scott's equation (Equation 45) for various solutions of electrolytes, sugars, alcohols, amide, and urea

$$ln\ a_w = \frac{-\Delta h_f}{R}\frac{(T_f - T)}{T_f T} + \frac{\Delta C_f}{R}\left[\frac{(T_f - T)}{T} - ln\left(\frac{T_f}{T}\right)\right] \quad (45)$$

where, T is the freezing point of solution, T_f is the freezing point of water, Δh_f is the latent heat of water, ΔC_f is the change of specific heat of water and R is the gas constant. The activity coefficient of water, γ_w was calculated using Equation (35).

Chandrasekaran and King (1971) used the two variable Margules equation and Equation (35) to predict the phase diagrams of multicomponent sugar systems using activity coefficients of binary sugar solutions. The limitation is the difficulty in attaining equilibrium due to crystallization of sugars in fruit juices that were investigated.

In a comprehensive analysis of concentrated sucrose solutions from 56 different studies ranging over a hundred years, Starzak and Peacock (1997) proposed a thermodynamically sound equation for the water activity coefficient based on Margules equation. The equation was validated using the data from all the studies. This model is based on the hydration of sucrose molecules and involves a significant number of constants. Water molecules are preferentially attracted to some solutes, including sugars, and are referred to as being hydrated. These molecules are not "active" and the water activity is correspondingly reduced (Starzak et al., 2000).

2.3.5.9. Solute activity

As has been seen it is possible to express water activity in terms of concentration and activity coefficients. Methods that can be used to calculate the activities of sugars in complex systems are as below. The relationship that best explains the concept of component activities is the Gibbs-Duhem relation (Hempel et al., 2012). The Gibbs–Duhem relationship can be applied to

a mixture of $c-1$ species, i, in solvent 1 (normally water) at constant temperature and pressure as below.

$$n_1 d\mu_1 + \sum_{i=2,c} n_i d\mu_i = 0 \tag{46}$$

Here n represents the number of moles of a substance and μ is the chemical potential which is a function of temperature, pressure and composition. The chemical potential can be related to the activity coefficient through Equation (47) (Long & McDevit, 1952).

$$\mu_i = RT\ ln\gamma_i x_i + \mu_i^{\circ} \tag{47}$$

Here μ_i° is the chemical potential at standard state, γ_i, x_i is the molar activity coefficient and the mole fraction of the solute. van Boekel (2009) used these fundamental principles and determined the solute activity of sucrose as shown in Figure 13.

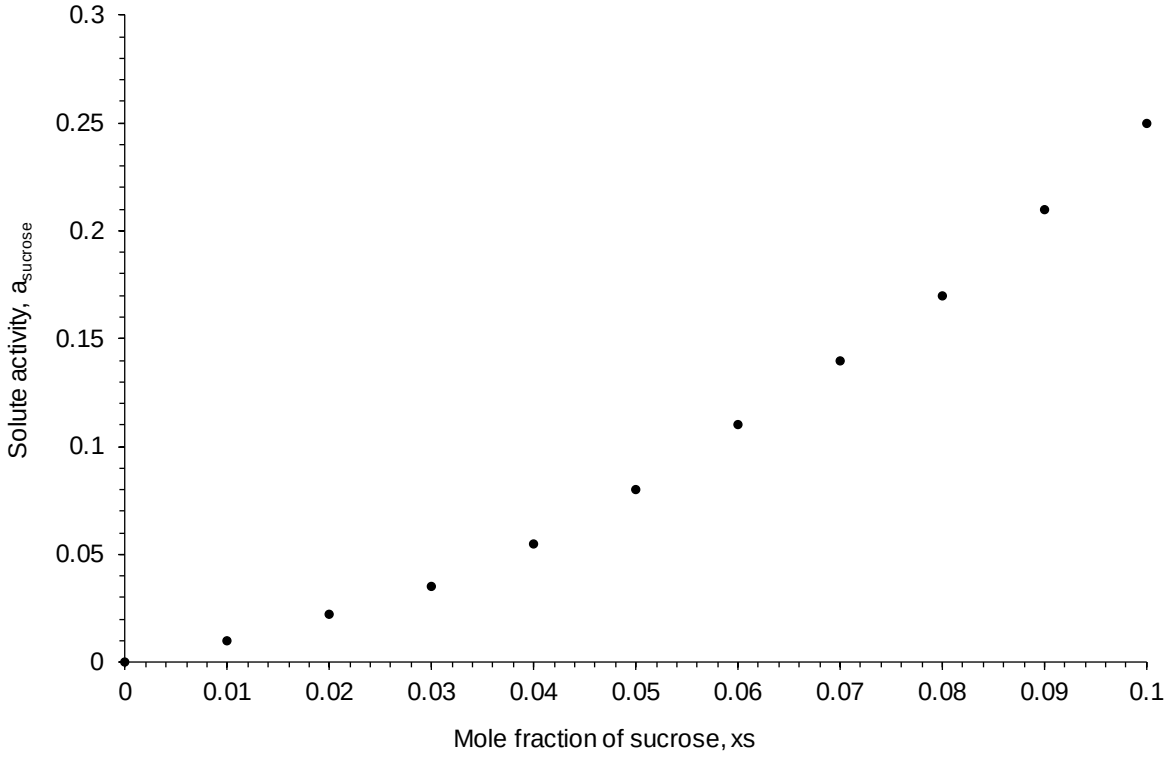


Figure 13 Calculated solute activity from the measured water activity data of a sucrose solution, van Boekel (2009).

Thus, a thermodynamic approach like the Gibbs-Duhem relation can be used to calculate the solute activity as shown in Figure 13. However, when a multicomponent solutions is considered with electrolytes and non-electrolytes, the application of the Gibbs-Duhem equation needs to

be considered. This leads us to extend Gibbs-Duhem to complex sugar systems with better understanding.

2.3.5.10. Hydration

For a binary system, the water activity can be related to hydration number, $\bar{n}$ using Equation (50) based on the approach of Scatchard (1921). The aqueous sucrose solution has been well researched and reviewed by Starzak et al. (2000). They gave a range of equations that have been applied to such similar systems. Water molecules are preferentially attracted to some solutes, including sugars, which are referred to as being hydrated. The molecules are not "active" and the water activity is correspondingly reduced. The hydration is dependent on concentration and temperature (Starzak, Peacock, & Mathlouthi, 2000). It was reported that although sucrose and water have the same active groups which are involved in establishing hydrogen bonds, the interactions between the molecules are very complex and the hydration of the sugar is specific for the conformation and configuration of the sugar (Lemieux & Pavia, 1969).

Table 5 Hydration number of mono- and disaccharides as calculated using the modified UNIFAC model (Gaida et al., 2006)

Sugars	Hydration number, $\bar{n}$
Xylose	1.33 ± 0.22
Fructose	3.39 ± 0.02
Glucose	1.93 ± 0.03
Galactose	1.81 ± 0.35
Maltose	4.48 ± 0.02
Sucrose	3.13 ± 0.02
Trehalose	5.80 ± 0.02

Typically, the hydration number of sucrose is about 5 to 6 while for glucose and fructose it is about 2. Saccharides are typical non-electrolytes with several hydrophilic hydroxyl groups, responsible for peculiar hydration characteristics in food systems (Banipal et al., 2002). Gaida et al. (2006) showed the variations in the hydration number using different measurement methods like viscosity, dielectrics, and simulations and also calculated hydration of simple sugars as shown in Table 5. While, a mono saccharide like fructose having hydration number around 3.4 and disaccharides like sucrose and trehalose having hydration numbers around 3.13 and 5.80 is in contrast to Starzak et al. (2000) approach which has values of 2 for monosaccharides and over 5 for disaccharides. This is the primary reason to have some

ambiguity about the use of group contribution methods in this study. Figure 13 shows the use of hydration number approach to eliminate the errors of water activity measurements. This effect is further discussed in the results section as the sensitivity associated with water activity measurements.

Alternatively, an equation (Equation 48) for the average hydration number was developed by Gharsallaoui et al. (2008) and is based on the change in density of the solution. This model is used as a tool for comparison and discussion but, will not be used any further than this. However it is evident and important to note the influence of volume of mixing and the effect it has on the calculation of the average hydration number.

$$\bar{n} = \frac{\Delta V}{\alpha_h \frac{w_s}{M_s}\frac{M_w}{\rho_w}} \tag{48}$$

$$\Delta V = \frac{m_s}{\rho_s} + \frac{m_w}{\rho_w} - \frac{1}{\rho_{sol}} \tag{49}$$

Here, α_h is the fractional volume reduction that occurs with hydration (0.092 for sucrose, 0.10 for glucose and 0.108 for fructose), w_s, w_w are the mass fraction of sugar and water M_s, M_w are the molecular masses of sugar and water respectively, ρ_{sol} is the density of the solution, ρ_s is the apparent density of sugar in solution, ρ_w is the density of water at the specified temperature and ΔV with units m^3/kg.

In this work the derivation from Scatchard (1921) as cited by Starzak et al. (2000) is used. It assumes that water molecules "attached" to the sugar molecules are not active, and are excluded from the concentrations used to determine the active mole fraction of water. This approach essentially defines hydration number in terms of water activity. Here superscript $^\circ$ refers to the nominal composition.

$$a_w = x_{w,active} = \frac{c_w^o - \bar{n}c_s^o}{(c_w^o + c_s^o - \bar{n}c_s^o)} \tag{50}$$

Dividing by total concentration, the concentrations can be converted into mole fractions, x. Here, x^o refers to the nominal mole fraction before considering hydration.

$$a_w = \frac{x_w^o - \bar{n}x_s^o}{(x_w^o + x_s^o - \bar{n}x_s^o)} \tag{51}$$

From this Scatchard's equation is obtained

$$a_w = \frac{\left(\frac{x_w^o}{x_s^o} - \bar{n}\right)}{\left(1 + \frac{x_w^o}{x_s^o} - \bar{n}\right)} \tag{52}$$

It can be rearranged as:

$$\bar{n} = \frac{x_w^o}{x_s^o} - \frac{a_w}{1 - a_w} \tag{53}$$

Given the water activity, the water activity coefficient, γ_w, can be calculated (a_w/x_w). Using this approach, the consistency of the hydration number is a very good indicator of experimental precision of water activity measurements, but at low solute concentrations the hydration number is very sensitive to small errors in water activity.

Starzak, Peacock and Mathlouthi (2000) used Equation (54) for sucrose with constants Q = – 17638 J mol^{-1}, b_1 = – 1.0038 and b_2 = – 0.24653. This equation gave the best fit to data up to about 95% by mass and temperatures from 0 to 148 °C. Equations (39), (52) and (53) together enable calculation of hydration numbers and the effect of temperature.

$$\ln(\gamma_w) = \frac{Q}{RT}(x_s^o)^2[1 + b_1 x_s^o + b_2(x_s^o)^2] \tag{54}$$

The existence of an equation for sucrose, implies the existence of similar equations for other sugars. Influence of sugars on hydration water can be described as affecting approximately three water molecules per each OH and thus, it appears that the complexity of the chemical structure of the sugars is not of primary importance. This further emphasizes the claims by Shimizu (2013) that a longer range of hydration region is present (Perticaroli et al., 2013). In another argument, Elamin et al. (2013) implies that water influences the dynamics of supercooled aqueous bulk solutions differently at very high and low water concentrations. The completely different structure and dynamics of water at high and low water concentrations further implies that different systems have different dynamics. Though, more complex theories exist such as these but for this work, Scatchard and Margules equation will be used on the basis

of comprehensive analysis of over 1600 data points by Starzak et al. (2000). Figure 14 shows the calculated values of average hydration number over a high range of concentrations at 25 °C using the Scatchard model.

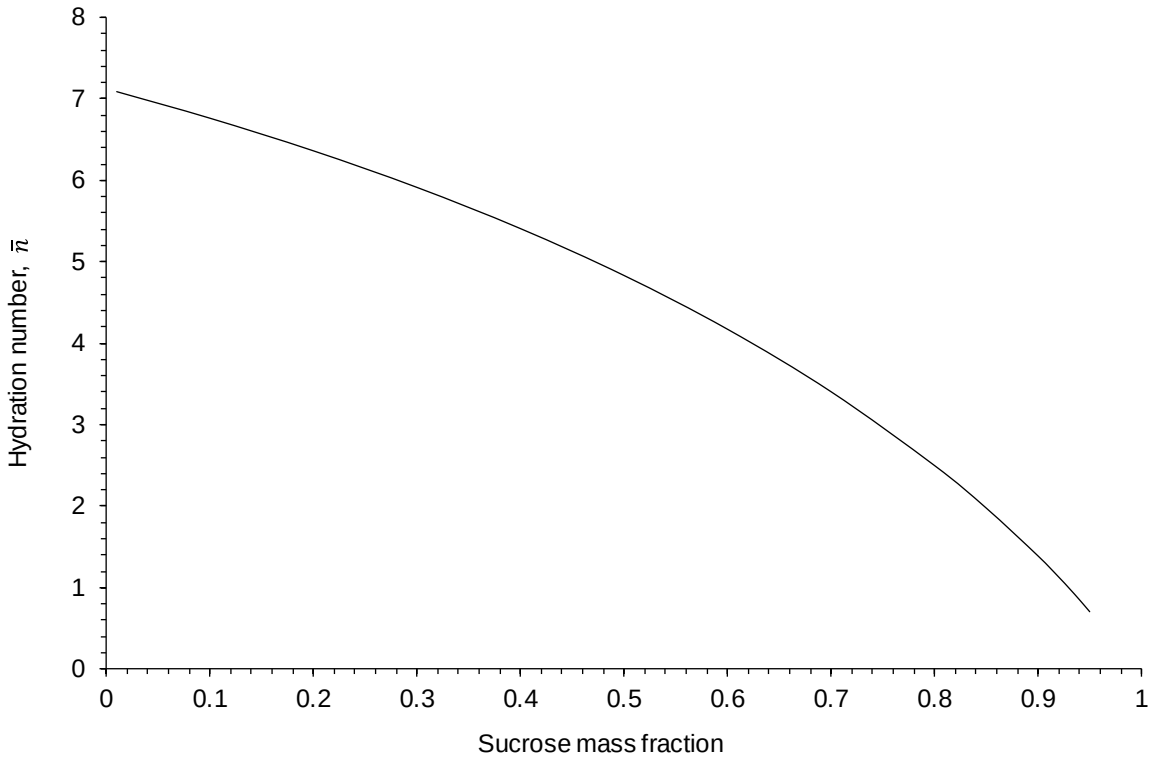


Figure 14 Predicted values of hydration number for binary sucrose solutions at 25 °C (Starzak et al., 2000).

2.3.5.11. Colligative Properties

Colligative properties of solutions are those that depend on the concentration of the solute and not on the characteristics of the solute (Hui, 1993). The fundamental thermodynamics of these colligative properties can be well explained using the Gibbs-Duhem equation (Williamson, 1967). Thermodynamic properties such boiling point elevation (Equation 55), freezing point depression (Equation 56) and osmotic pressure (Equation 57) are known as colligative properties. The equations below are the colligative properties of solutions expressed in terms of water activity as given by Berry et al. (1980).

$$ln(a_w) = -\frac{\Delta h_{1v}(T - T_{1b})}{RT_{1b}^2} \tag{55}$$

$$ln(a_w) = -\frac{\Delta h_{1f}\left(T_{1f} - T\right)}{RT_{1f}^2} \tag{56}$$

Higher orders of Equations (55) and (56) were omitted as these seemed to be insignificant during analysis. Here, Δh_{1b}, Δh_{1f} are the latent heat of vaporisation and freezing respectively,

$$\varphi = \frac{\ln(a_w)}{vmM_A} \tag{57}$$

where φ is the osmotic coefficient, v is the number of ions produced when a solute is dissolved, and R is the universal gas constant.

The effect of water in complex sugar systems in general needs to be understood better from the point of solutes and their chemical structure. Deviation from ideality of solutions should be understood and validated. Properties like freezing point (depression), boiling point (elevation) have deviations and this can be attributed to the interactions between the numerous sugar molecules and the little water molecules. Zavitsas (2010), Slade and Levine (1991), Wang et al. (2016) all explain the significance and effects of these deviations known as the colligative properties of solutions.

2.3.6. Mutarotation Studies

Nelson and Beegle (1919) observed a chemical reaction in the solution state of sugars, which contributed to changes in the physical properties of these sugar systems known as mutarotation. Sugars like glucose and fructose change their optical rotatory powers in solution due to transformation of the substance from one form to another. This phenomenon is known as mutarotation. Ever since this discovery, scientists have observed this phenomenon and accumulated data on the same. Temperature affects the mutarotation kinetics and equilibrium in a system. Figure 15 and Figure 16 shows the different schemes of mutarotation of glucose and fructose.

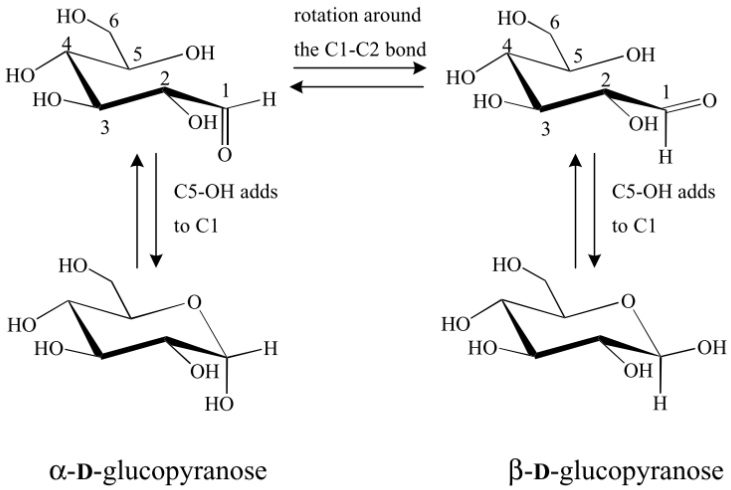


Figure 15 Mutarotation schemes of glucose

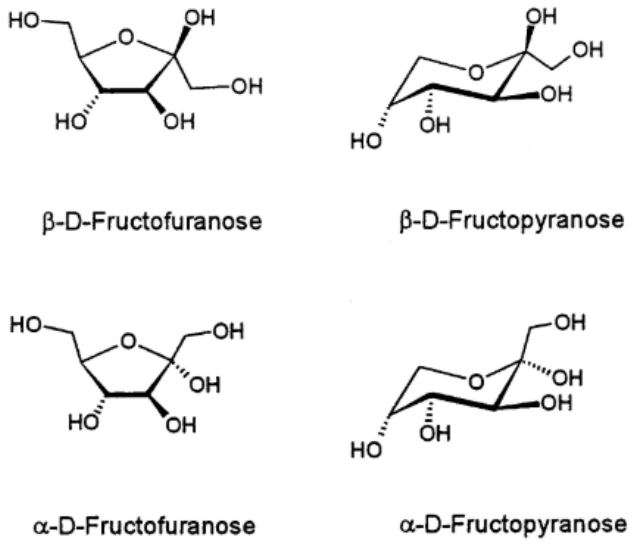


Figure 16 Mutarotation schemes of fructose

Though mutarotation is accounted for in physical property measurement for simple to complex sugar systems, the effect of mutarotation on crystallization is something that needs to be looked into in detail. Van Hook (1961) discusses the effect of the conversion of α to β forms of glucose and the effects these cause on the crystallization of glucose. Kraus and Nyvlt (1994) also discussed similar effects as only the α form of glucose crystallises, and when it does, some of the β form is converted to the α form to re-establish equilibrium.

Van Hook (1961) concluded that the rates of crystallization and solution of these and other sugars which exist in several isometric forms are controlled entirely by the rate of conversion of one form into the other Srisa-nga and Flood (2004) concluded that the mutarotation reaction may complicate the crystallization kinetics and it is necessary to study its kinetics as well as equilibria. Flood and Srisa-nga (2012) developed a crystallization model that incorporated the effect of mutarotation reaction on the crystallization of glucose monohydrate. Hence, an effort is made in this study to understand the interaction between mutarotation and other properties of model food systems.

2.3.6.1. Nuclear Magnetic Resonance (NMR)

As the primary focus is on sugars, details about Nuclear Magnetic Resonance (NMR) technique are discussed as applied to sugar systems to measure mutarotation. The effect of temperature and concentration of the sugar solutions (glucose and fructose) was determined using the NMR technique by Hyvonen et al. (1977). The inference from this study was that both concentration and temperature affected the mutarotation of these sugars. At higher temperatures and higher concentrations of glucose solutions, the α- glucose anomer has a higher concentration.

The mutarotation of D-fructose in aqueous-ethanolic solutions was studied between 24 and 50 °C at different ethanol:water mass ratios. The principal tautomers found in the solution were β-D-fructopyranose, β-D-fructofuranose, and α-D-fructofuranose, as are found with aqueous solutions of D-fructose. The rate of mutarotation from β-D-fructopyranose to the furanose tautomers was five-fold slower in solutions of ethanol:water ratio of $9:1$ than in aqueous solutions. The tautomeric composition of D-fructose in aqueous-ethanolic solutions was shown to affect the crystallization of the sugar, due to the slow rate of mutarotation of furanose forms to β-D-fructopyranose (Flood et al., 1996). Figure 17 shows a typical mutarotation behaviour of glucose in aqueous solution as measured by NMR technique.

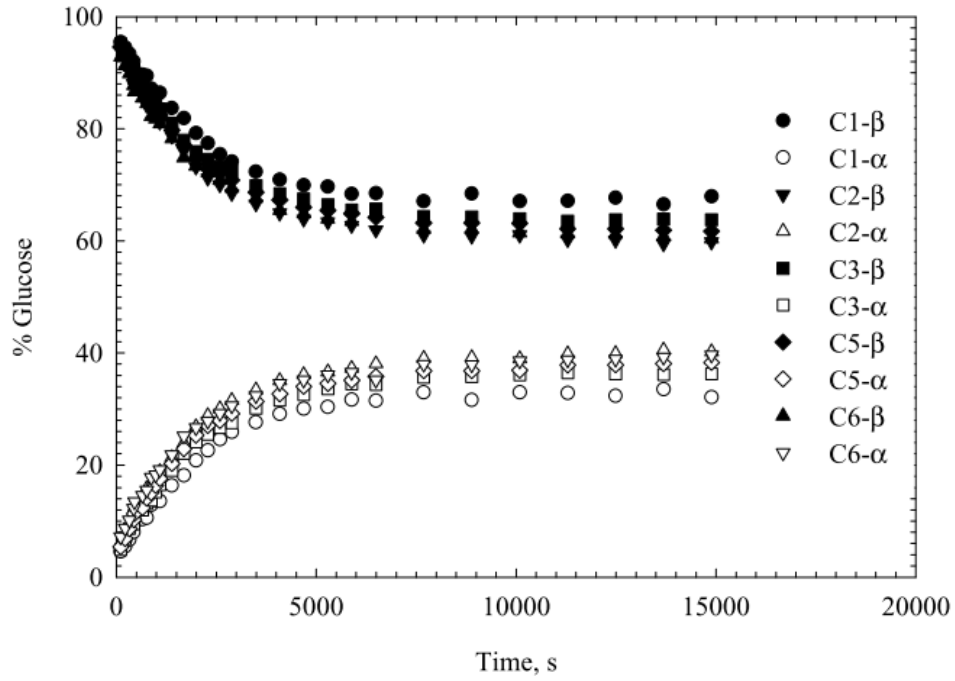


Figure 17 Mutarotation behavior of glucose in aqueous solution at 24 ºC. Note that zero time corresponds to the starting of the NMR measurement, not to the initial mixing time (Srisa-nga, 2005).In the legend 'C' refers to the carbon atom to which the functional group is attached.

In the study by Jawad et al. (2012) on lactose mutarotation, β/α anomer contents of amorphous lactose measured by 1H-NMR had standard deviations as low as 0.1% w/w (n = 6). Drying a lactose solution 4 hour after its preparation led to almost 35% w/w difference in anomer composition within solid amorphous material compared to samples dried after only 30 min, e.g. for freeze dried samples, β content was $60 \pm 0.1\%$ w/w (4 h) and $25 \pm 1.0\%$ w/w (30 min). Mutarotation leads to this increase in β anomer concentration in aqueous solution and within the solid amorphous lactose stored at 25 ℃ e.g. after 56 days storage the β content of freeze dried lactose (30 min solution) increased from $25 \pm 1.0\%$ to $50 \pm 0.5\%$ w/w.

2.3.6.2. Polarimetry

Optical rotation is a phenomenon where a medium rotates white right circular polarised light clockwise or anticlockwise (Capon & Overend, 1960). Though there are different techniques available to measure the mutarotation, optical rotation measurement using a polarimeter, used as a control for the NMR experiments in this work.

A comprehensive study on the optical rotation of mixtures of glucose, fructose and sucrose by Vosburgh (1921) showed some key findings as summarized.

- The optical rotations of an invert sugar mixture is the algebraic sum of the optical rotations of glucose and fructose solutions on their own.
- Similarly, mixtures of glucose and sucrose showed the same cumulative effect as shown by invert sugars.
- However, for fructose and sucrose mixture the rotations were contrary to the above and the rotation values were a little smaller. Hence, glucose was chosen for NMR studies.
- Salts and acids in low concentrations did not affect the optical rotation of sugars.

Stevens (1993) proposed a calculation model to predict the optical activity of a series of sugars based on Kirkwood's polarizability theory of optical activity. Lo Nostro et al. (2006) showed the effect of salts on the optical activity of glucose solution. However, further discussion on the reasons for reduction in the optical activity due to addition of salts at concentrations over 3 mol/L solution was not provided satisfactorily. Soetedjo and Räty (2014) used the modified Drude's equation to explain optical rotation in sugars and also showed the variation of optical activity due to different wavelengths of the light source.

A key aspect to note is that literature was unavailable to establish the effect of viscosity on the mutarotation of the concentrated sugar solutions. This study will try to establish an understanding of the effect of viscosity of concentrated sugar solutions on mutarotation.

2.4. State of Water in Food Systems

Water plays a major role as a solvent in the properties of a solution. Water dissolves most sugars from or in food materials that are available in nature. A single water molecule is in the form of a cluster with up to 4 other water molecules surrounding that single molecule in water and ice. Water molecules form a number of hydrogen bonds amongst themselves and these influence to the properties exhibited by solutions with water as the solvent. The association of water molecules with each other and with the solute needs to be studied to understand the physical properties of solutions.

The properties of solution must vary based on multiple interactions like the water-solute interaction, solute-solute interaction and the water-water interactions. The importance of solute-solute interactions conversely support the theory of solute hydration and clustering

which advocates the interplay of solute-solute and solute-water interactions (Maneffa et al., 2017). The effect of hydrated solute can be incorporated into the mass fraction of the solute. The decisive role of free water helps establish the relevant properties of aqueous solutions (Wang et al., 2016). The impact of concentration on the environment of a glucose molecule and the formation of cages made by neighbouring glucose molecules at higher concentrations were correlated to the spin and rotational decoupling diffusions of water (Smith et al., 2014). The dehydrated trehalose matrix is homogeneous in terms of distribution of the residual water and spin-probe molecules. In contrast, dehydrated sucrose forms a heterogeneous matrix. It is comprised of sucrose polycrystalline clusters and several bulk water domains (Malferrari et al., 2014).

Water in a system can generally be considered to be “free” water or “bound” water as illustrated in Figure 18. The terms “free” and “bound” have many different definitions. In simple terms, the most highly “bound” water can be described as the water that cannot be dehydrated from any molecule (Rodel, 1993). “Free” water may be defined as the water that can be frozen in a system. “Free” water in a system can further be in an associated or non-associated form. The associated “free” water may be responsible for molecular degradation or ionic activity. The “free” water can also be called bulk water in further references.

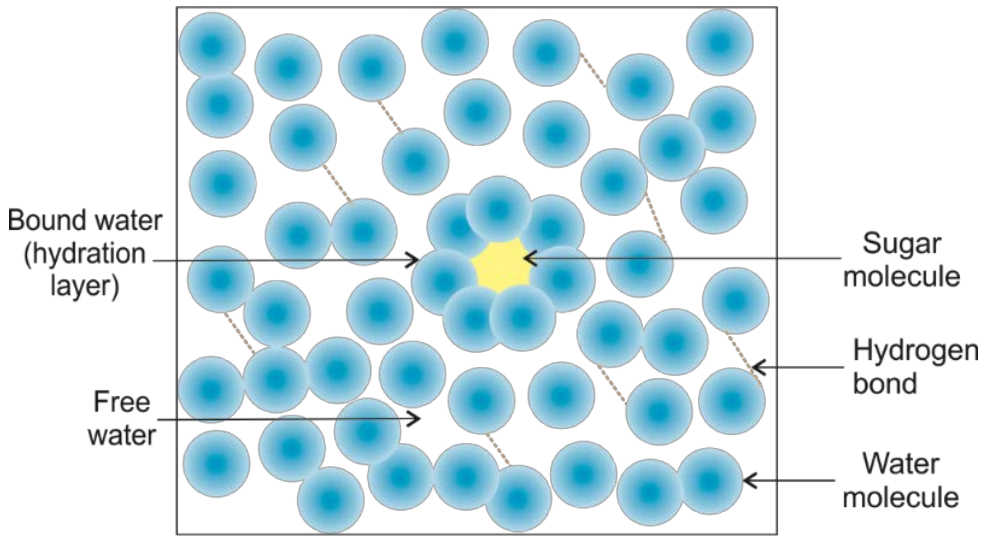


Figure 18 Illustration of bound and free water.

As discussed earlier in this section, water exists in different forms in food systems. Figure 19 shows the different forms of water in a food system.

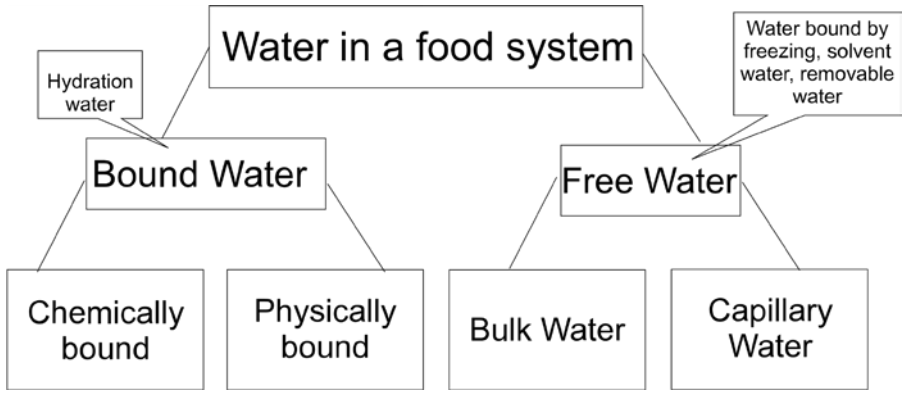


Figure 19 Types of water in foods. The image is an indicator of all the types of water in foods.

2.5. Crystallization

Crystallization is common in food products containing concentrations levels above saturation (Zamora & Chirife, 2006). Supersaturated sugar solutions tend to crystallize due to the limiting solubility of the sugars and the scarcity of solvent present in the system.

Hartel (2013) described the four stages that make up the process of crystallization:

- Super saturation: Super saturation creates a driving force for crystal formation from the liquid phase and overcomes the crystallization energy barrier. Once this has been achieved, nucleation can occur.
- Nucleation: Nucleation involves the transition of liquid state (like honey) molecules to stable nuclei and crystalline lattice structures
- Crystal growth: molecules can then deposit onto the existing lattice by diffusion for crystal growth. This occurs until the driving force for crystallization is reduced to zero
- Recrystallization: Recrystallization involves a change to the number, shape, size or orientation of crystals post initial formation and usually occurs during storage

The rate of glucose crystallization can be affected by a number of factors, including by water content, presence of nucleation seeding, degree of supersaturation, glucose diffusivity, viscosity, temperature and saturation solubility (Venir et al., 2010). Both glucose diffusivity and saturation solubility are temperature dependent (Al-Habsi et al., 2013). An increase in

temperature can cause the solubility of glucose to increase, resulting in a lower tendency for crystallization. However, a decrease in temperature lowers the glucose diffusivity and slows crystallization (Al-Habsi et al., 2013). As a result, an optimum crystallization rate can be achieved at temperatures between 10 and 15 °C (Zamora & Chirife, 2006).

As honey is a supersaturated glucose solution, it can spontaneously crystallize at room temperature to form solid glucose monohydrate crystals. The crystallized mixture exists in a metastable, or thermodynamic non-equilibrium state (White Jr. & Doner, 1980). In honey similar to other foods, it is critical to get the precise size distribution, phase volume, morphology and so on to achieve required product quality (Hartel et al., 2011).

The primary problem of crystallization in honey is a consumer's perception of texture with changes in viscosity and mouth feel. The secondary problem of crystallization is it also increases water activity of honey which can lead to fermentation of any naturally occurring yeasts; further explained in Section 2.5. As a result of fermentation, honey products can adapt a sour taste or cloudy coloration (Zamora & Chirife, 2006) and cannot be sold to the consumer. While the behaviour of honey crystallization is difficult to predict, an understanding is imperative for production of high quality, consumer-ready products with an extended shelf life (Hartel, 2013).

2.5.1. Diffusion

One key parameter that takes precedence when we discuss physical properties like water activity, hydration and crystallization is diffusion. Diffusion in a bulk solutions, diffusion of water within a system which includes both bound and free water and diffusion of sugars or additives in a system needs to be considered to understand some of the physical phenomena.

The processes of nucleation and crystallization are essentially diffusion controlled as stated by Van Hook (1969). Hartel and Shastry (1991) summarized some parameters that might be controlling crystallization and they included diffusion (surface diffusion, counter diffusion of water), mutarotation, and orientation of sugar molecule. Gharsallaoui et al. (2008) showed that the crystallisation of anhydrous disaccharides in aqueous solution causes the release of

hydration water and the diffusion of this water from the crystal surface to the bulk solution is the controlling step of the crystallisation process.

Starzak and Mathlouthi (2002) explained the diffusion occurring in the film surrounding a sugar crystal. As an inference, the activity of the water in a food system with sugar depends on the sugar concentration, sugar diffusion and sugar crystallization. The diffusion of sugar in aqueous solution is important both in nature and in technological applications, yet measurements of diffusion coefficients at low water content are scarce. Magazu et al. (1998) discussed the effects of diffusion and viscosity on sugar crystallization (Figure 20). At lower volume fractions of trehalose, there exists a repulsive nature among trehalose molecules as represented by the dashed lines and as the volume fraction increase this becomes a cooperative zone which shows the hydrodynamic interactions. This trend is shown for temperatures above 40 ºC where the diffusion reaches a maximum and decreases thereafter. It was observed that the growth-rate of crystals was reduced where crystallization was partially dependent on diffusion in fructose crystallization (Flood et al., 2000).

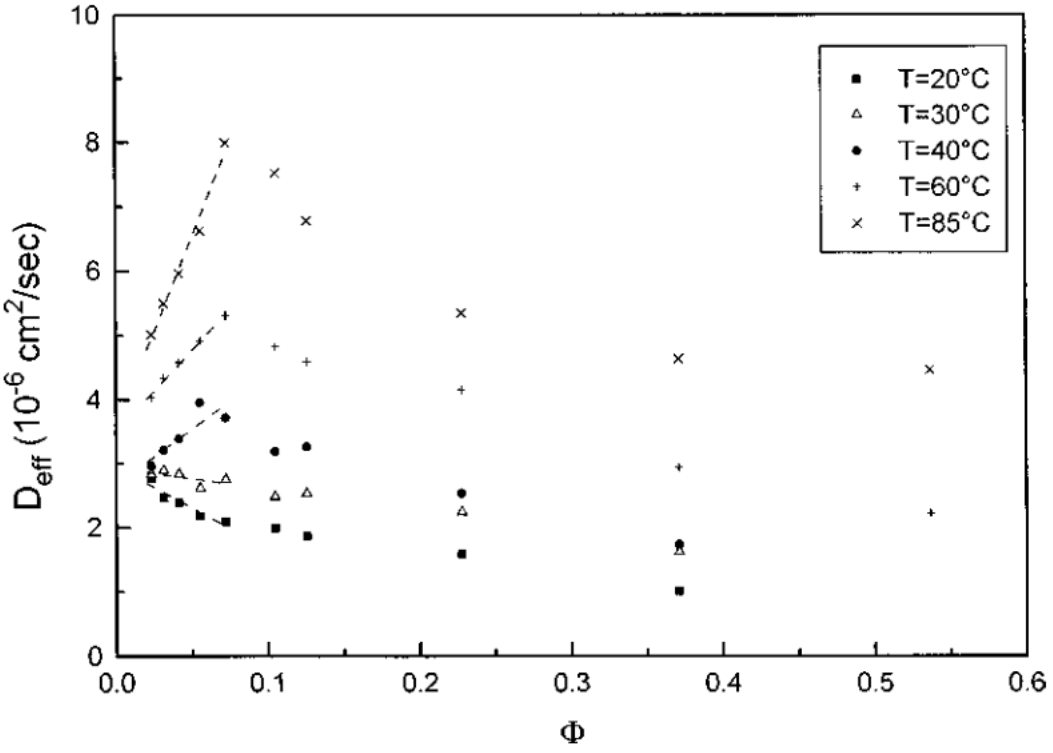


Figure 20 Effective diffusion behavior as a function of volume fraction at different temperature of trehalose. The dashed lines represent the initial repulsive nature of trehalose (Magazu et al., 1998).

Price et al. (2016) calculated the sucrose diffusion coefficient in a solution with a water activity of 0.4 (equivalent to 90% sucrose by weight) using Raman isotope tracer method by monitoring the diffusion of non-deuterated and deuterated sucrose across a boundary between the solutions and concluded that the diffusion coefficient of sucrose was approximately four orders of magnitude smaller than that of water in the same material. The Stokes–Einstein equation worked well for predicting sucrose diffusion using some viscosity data from literature.

Chenyakin et al. (2017) used the rectangular area fluorescence recovery after photobleaching (rFRAP) technique to measure the diffusion coefficient of fluorescent dyes in sucrose–water solutions with various water activities of 0.38 to 0.80. The Stokes-Einstein Equation was tested in these systems and was in agreement with the diffusion of dyes however, there were some discrepancies in the water diffusion coefficients of sucrose solutions with high water activities. This might be due to not introducing the correction factor α like in the modified Walden's equation (Equation 10).

Near solid foods that are rich in sugar are also referred to as a "biological matrix", for example, a strawberry or a red onion. When a "biological matrix" like a strawberry was analyzed using broadband dielectric spectroscopy, the microscopic dynamics of associated water were shown. The water molecules interact more strongly with carbohydrates and other biological materials at low water content. The conductivity of these "biological matrix" is only from long-range diffusivity at temperatures greater than 250 K (Jansson et al., 2005) for reasons like, (a) an almost rigid matrix where the water is unable to perform long-range diffusion due to confinement effects, (b) a dynamic matrix with no static confinement effects.

2.5.2. Solubility

The solubility of a sugar primarily depends on the temperature of the dissolution. Solubility of a sugar increases with increasing temperature. The solubility of a particular sugar is affected by the presence of other sugars. The solubility of sucrose is considerably reduced by the presence of invert sugar in a mixture of the sugars. Similarly, fructose reduces the solubility of glucose in a mixture containing glucose and fructose (Jackson & Silsbee, 1924). Hence, to understand a system like honey the understanding of glucose solubility is important.

Kelly (1954) studied solubilities of sugars at different temperatures in mixtures like water + glucose + fructose, water + glucose or fructose + KCl, water + sucrose + glucose or fructose and water + sucrose + KCl and concluded that the solubilities of a particular sugar or salt influenced the solubilities of the major component in the system and thus causes the "salting-out effect" which is essentially crystallization.

Solubility of sugars in a food systems can be modelled and predicted using the Flory-Huggins theory over a wide range of temperature and concentration (van der Sman, 2017). Similar models can be applied to measure the solubilities in case of multicomponent sugar systems to predict the crystallization behaviour. Solutions with the sugar contents higher than the solubility concentration are called supersaturated solutions (Hartel & Shastry, 1991). These supersaturated solutions are necessary for the crystallization of sugars. The thermodynamic expression of chemical potential of a sugar in solution, at equilibrium can be expressed in terms Equation (58)

$$\mu_{sugar\ in\ liquid} = \mu_{solid\ sugar} \tag{58}$$

2.5.3. Glass transition temperature

Bhandari et al. (1999) described the temperature at which the reaction rates in a solution is increased rapidly as the glass transition temperature, T_g. Roos and Karel (1992) concluded, based on their study on lactose crystallization, that the $T - T_g$ determines the rate of crystallization. Above 30 ºC, crystallization of honey is nearly zero due to the increased solubility of glucose. Some models are built around understanding the crystallization rate based on the glass transition temperature (Bhandari et al., 1999). Roos (2010) concludes that glass transition temperature is the factor for food stability, food structure modifications and component crystallization. Roos' work acknowledges the contribution of glass transition towards crystallization, but does not measure values of glass transition.

2.5.4. Sugar ratios

Table 6 shows the various crystallization indicators used in the honey crystallization industry (Bhandari et al., 1999). These include water content (W), glucose/water (G/W) ratio, fructose/glucose (F/G) ratio, glucose-fructose/fructose $\frac{G-F}{F}$ ratio. The Tabouret index (I) is

based on the glucose water (G/W) ratio and the measured water activity as given in Equation (59). Here, n is a constant that depends on the moisture content. If the moisture is $< 17\%, n = 1$ and when the moisture is $> 17\%, n = 2$.

$$I = \frac{\frac{G}{W}}{(1 - a_w)^n} \tag{59}$$

Tosi et al. (2004) proposed another indicator $(\text{fructose/glucose}) \times \text{moisture}$ that can be used to predict crystallization. Conforti et al. (2006) concluded that there existed no relationship $(\text{glucose} - \text{moisture})/\text{fructose}$ as a parameter that is usually used to predict crystallization at room temperature. However, found that some samples showed a lower tendency to crystallize when using the relation $\text{moisture} - (\text{fructose/glucose})$.

Table 6 Crystallization indicators used for honey (Bhandari et al., 1999).

Indicators	No granulation	Fast granulation
G/W ratio	< 1.70	> 2.16
F/G ratio	> 1.33	< 1.11
$\frac{G-F}{F}$ ratio	< 0.30	> 0.49
% Glucose	< 27.7	> 35
Coefficient of super saturation of glucose	< 1.8	> 2.6
Tabouret index	< 9.8	> 12.6

Granulation is considered as a stage in crystallization and hence it is used above. Though these indicators help to predict crystallization behaviour of sugars in the honey business, there is no thermodynamic basis. Given the fact that these empirical indicators ignore the chemical potential of the solute it is not surprising that they are not always reliable. However, to understand the effect of these parameters in a multicomponent system, some experiments were carried out in this work.

2.5.5. Seeding

Active sites are required for the initiation of crystallization. Seeding provides the nucleus or the active sites for the onset of crystallization in sugar systems. Seeding is the process of placing seed crystals (small pieces of crystal on which a large crystal of the same material will grow) in a supersaturated solution. This causes crystallization to occur and ideally enables the structure and shape of the crystals formed to be controlled. Markande et al. (2012) indicated that seeding mass increases crystallization. However, the size of the crystal seed, the amount

added and the cooling profile influenced the crystal size and yield of glucose monohydrate. Similarly, while studying the effect of mutarotation on fructose crystallization, Flood et al. (1996) showed that seeding increased the crystal growth rate.

2.5.6. Additives

Doremus (1984) found the presence of acids, salts and other sugars reduced the crystallization of sucrose by decreasing the viscosity and hence increasing the diffusion coefficient. In the review on the crystallization of food products, Hartel and Shastry (1991) concluded that "*the presence of impurities and additives (including mixed sugar systems) affects both the nucleation and growth steps*". Mazzobre et al. (2001) observed similar characteristics of lowering in the crystallization of water with addition of salts and this is attributed the solvation characteristics of water.

Longinotti et al. (2002) studied the crystallization of trehalose and sucrose under the influence of salts and concluded that the very high nucleation rate as the cause of lower crystallization rates for sucrose systems and that salts affected the temperature dependence. Chandrapala et al. (2016) confirmed similar effect of lactic acid and calcium on the crystallization of lactose in a model lactose system. Hence, further study was required in a complex mixture of sugar system like honey and this work had a focus on this.

2.5.7. Model food systems with sugar

Model honey systems were developed based on the principal components in honey. Fructose and glucose, being the major sugar component in any type of honey, were used as the principal components. Sucrose was used to understand any variations due to higher sugars. Stinson et al. (1960) mentioned that the primary acid present in honey is gluconic acid along with other acids like malic acid. Malic acid was used in model honey systems to replicate the pH of a typical honey sample. Similarly, sodium chloride was added in trace quantities as the contribution of all these components would replicate honey properties.

Rüegg and Blanc (1981) obtained water activity measurements of model honey solutions using an electronic hygrometer. They used water fractions from 12.1% to 28% with a dry-basis composition of 48% fructose, 40% glucose, 10% maltose and 2% sucrose. This model can be

compared to that of honey and complex sugar system. Equations will be developed based on the water activity data from the paper and will be compared with the equation from experimental data.

Another food system was a quaternary system with different sugar is apple juice reported by Fontan et al. (1981) with typical composition of 62.5% fructose, 14.7% glucose and 22.7% sucrose mass fraction. This system was further tested by Velezmoro et al. (2000) to predict the water activity of the solution using group contribution models.

2.6. Chapter Summary

This chapter detailed all the key research finding and data available in the literature on the physical properties of sugar systems. All the fundamental relations and models were discussed in detail for each physical property. The inter relation of these properties need to be understood for a thermodynamic basis to derive equations and create newer models. Areas with need for further understanding were identified and will be addressed in the methods and results section

3 MATERIALS & METHODS

Almost all experiments were carried out at controlled temperature condition of 20 °C unless specified. This chapter is sub-divided into materials and methods. Detail description of all the materials that were used during experimentation is listed. Solution preparation and experimental methods are discussed in detail with adequate detail. All experiment measurements were triplicates except crystallization experiments. Adequate time was not available to repeat crystallization experiments. The results are still presented to allow discussions of these results.

3.1. Materials

A local food ingredients company, Formula Foods, provided glucose (dextrose monohydrate) and fructose, while sucrose (William's Tea and Coffee, NZ) was purchased from a supermarket. The salts content of the sugars was not critical as this was determined by analyzing electrical conductivity results. Malic acid (makewine.co.nz) being an easily available weak organic acid was used in this study. Deionised Milli-Q water was used for all solution preparation and cleaning of the conductivity cell. Each sugar component was analysed using high performance liquid chromatography (HPLC) in order to establish the purity and in each case a single peak was obtained with a resolution of better than 0.1%. Laboratory grade sodium chloride and potassium chloride were purchased from Biolab, Australia and Thermos Fischer Scientific respectively. The sodium carboxymethyl cellulose (NaCMC) was so purchased as Walocel C (DOW). The protein content of the whey protein isolate (WPI) was 92% by mass. The characteristics of each substance used in this experiment are outlined in Table 7

Table 7 Chemicals source and purity (mass basis).

Substance	Purity	Provider
Sucrose	> 99.9%	Williams White Sugar
D-Fructose	> 99.9%	Formula Foods
D-Glucose monohydrate	> 99.9%	Formula Foods
NaCl	99%	Biolab Ltd, Aus
KCl	99.8%	Thermo Fischer Scientific
Malic Acid	> 99.9%	Makewine.co.nz
Sodium CMC	99.5%	Walocel C, DOW
Skim Milk Powder		Dairyworks, NZ
Maltose Monohydrate	≥99%	Sigma-Aldrich
Lactose	100%	ECP Lab Chemicals
WPI	92%	Balance Sports Nutrition, NZ

3.2. Methods

Experimental methods were followed in each step with the utmost priority given to precision of work. Precision and accuracies of physical instruments were maintained throughout the duration of this research work. Calibrations were performed on every instrument prior to start of a series of experiments along with periodic calibration. Apart from devising a clear and precise methodology, elimination of all sources of inaccuracies was attempted. For instance, it was found that the measured mass of the polystyrene LabServ containers was more stable if they were kept open for 12 hours prior. In the process of solution preparation, double checking of masses showed that a balance drifted while under-weight so this balance was replaced. Wherever possible the refractive index, which was found to be repeatable, was used to confirm the solids content.

3.2.1. Solution Preparation

All sugar or standard solution was prepared with a standard 250 mL LabServ clear polystyrene container using a Sartorius R300S balance with a precision of 0.1 mg. The LabServ containers were kept open to equilibrate with the environmental conditions for 12 hours. All measurements were performed after 24 hours of preparation unless mentioned. Finally, to confirm complete dissolution, a laser beam was passed through the solution to confirm no near scattering (Tyndall effect) of the beam took place. All sugar solutions were prepared using Milli-Q water with resistance specified as 18.2 MΩ at 25 °C. Solution concentrations are specified as mass fractions unless specified.

3.2.1.1. Binary solutions

The desired weight of the solution usually used was 100.0000 g for convenience. An equilibrated standard 250 mL LabServ container was placed on the balance and the balance was tared when a stabilized weight was read on the screen. Then the calculated weight of sugar was added to the container. After a few seconds of stabilization, fresh Milli-Q water was added up to the display weight close to 99.9900 g using a wash bottle. The remaining weight of water to make-up to the desired 100.0000 g is filled using a micropipette. For sugars with monohydrates associated, adequate calculations were made as the monohydrate increased the

mass of sugar to be weighed and reduced the amount of water that needed to be added to form the desired solution. The solutions thus prepared were then incubated in an IKA Incubator KS4000i at a speed of 165 rpm and a temperature of 60 °C for 2 hours or longer based on updated methods of preparation under careful consideration on decomposition of sugars. Preliminary experiments were performed at a speed of 130 rpm (revolutions per minute) and a temperature of 55 °C for 1 hour with the methods as proposed in Gomez-Diaz et al. (2009) as the processing condition for removing crystals in honey. The solutions were then allowed to cool down to room temperature for 12 hours before measurement. The expected precision of the solutions were within $\pm$ 0.5 mg.

3.2.1.2. Model honey solutions

Model honey solutions were prepared in the same manner as binary solutions. When there were more than two components, the smallest by weight was added first in order for any weight corrections to be made. Components like salt and acid are added in minute quantities and hence measured using a weighing paper and added into the tared empty container followed by sugars directly. Model honey solutions were prepared based on the average floral honey composition as determined by Doner (1977). The base composition that was used for the model honey solutions is as outlined in Table 8 below. All model honey solutions were incubated at a speed of 165 rpm and a temperature of 60 °C for 8 hours. The solutions were then allowed to cool down to room temperature for many hours before measurement.

Table 8 Model honey solution composition (Doner, 1977).

Water mass fraction %	Fructose mass fraction %	Glucose mass fraction %	Sucrose mass fraction %	F/G ratio	S/G ratio
17	45	36.5	1.5	1.23	0.041

3.2.1.3. Invert sugar / multi-component invert sugar solutions

The principle underlying the preparation of invert sugar solutions was similar to binary solutions. Equal quantities of glucose and fructose after accounting for the monohydrate were measured in the balance and fresh Milli-Q water was added to the desired weight of the solutions, which was usually 100.0000 g. All invert sugar solutions were incubated in IKA Incubator KS4000i at a speed of 165 rpm and a temperature of 60 °C for 2 hours. The solutions were then allowed to cool down to room temperature for 12 hours before measurement.

3.2.1.4. CMC solutions

Sodium carboxymethyl cellulose (NaCMC) solutions were prepared at concentrations up to 1% by mass of NaCMC (wet basis) using a homogenizer. The NaCMC was found by oven drying to contain 14.8 % free moisture. In addition various concentrations of KCl, NaCl and sucrose were added. Solutions of salt + sugar + NaCMC were prepared by adding measured quantities of solids and water followed by dispersion of solids by using a stick homogeniser (Yellowline DI 18 basic). Furthermore the prepared solutions were allowed to further hydrate for 12 hours at 20 ºC and 160 rpm. Solutions of whey protein isolate (WPI) (Balance Sports Nutrition, New Zealand), with and without lactose and NaCl were also prepared.

3.2.1.5. Skim milk solutions

Skimmed milk solids purchased from a local supermarket (Dairy works, NZ) was used to prepare milk solutions. The composition was given as 92% WPI and the balance contained sugars, salt and fat. A homogenizer was used to disperse the milk solids in Milli-Q.

For solutions containing up to 20% solids, the milk powder was added to water and dispersed for 5 minutes followed by incubation for 40 minutes at 45 °C. For solutions with milk solids above 20% and below 30%, 10 minutes of dispersion with the homogenizer along with manual movement of the container in an alternating circular and vertical motion.

For all solutions above 30% solids, the same procedure as for $20\% - 30\%$ solids was followed. For solutions below 30% solids it was possible to observe the effectiveness of dispersion, but above this level it was more difficult and samples were left at room temperature for a further 6 hours after the incubation time to ensure near complete hydration.

3.2.2. Measurement

3.2.2.1. Density

Densities of the sugar solutions were measured using an Anton Paar density meter maintained at 20 ± 0.002 °C with help of a water bath and a Fluke temperature controller. The density

meter works on the principle of measuring the frequency of a vibrating U-tube. It was calibrated each day with water and air.

3.2.2.2. Electrical Conductivity

The electrical conductivity (σ) of the solutions were measured using a Philips PW951C conductivity cell (with parallel platinum electrodes) connected to a FLUKE PM6306 programmable RCL meter set at 100 mV a.c.. Additionally, due to damage to the Philips sensor, a Schott LF413T probe was connected to the RCL meter with custom made connectors for all measurements. A custom-made sample holder was used to seal the conductivity probe in place and to provide consistent experimental conditions. The container was immersed in a water bath with temperature control of ± 0.02 °C.

The temperature was measured with two precision platinum thermometers to an accuracy of ± 0.01 °C. The RCL meter was controlled by a data logging unit to obtain measurements of the impedance, Z, resistance, R, and the phase angle at 26 pre-set frequencies ranging from 100 Hz to 1 MHz. After measurements at different frequencies the resistance at any frequency with zero or near zero phase angle was selected for the conductivity calculations. The specific conductance or conductivity, σ, of the solution was calculated from the resistance using Equation (60).

$$\sigma = \frac{Resistance(R)}{Cell\ constant\ (l/A)} \tag{60}$$

The cell was calibrated using standard KCl solutions with different mass fractions, and cell constants were determined periodically. In addition, the electrical conductivities of the KCl solutions were also measured using a calibrated Schott ProLab 970 conductivity meter (SI Analytics, Germany) as a control. All conductivity values measured or calculated using the measured resistance were corrected to 20 °C using a measured temperature co-efficient of 0.020 °C^{-1}.

This conductivity study was conducted to establish a relationship for a range of food liquids at higher concentrations and hence higher viscosities. Model solutions were prepared using sugars, whey protein, milk powder, carboxymethyl cellulose and salts, and the electrical conductivity and viscosity of these was measured. A relative error with calibration was within

$\pm$ 0.01 µS/cm. Similarly, DI water after handling during solution preparations was within + 0.5 µS/cm.

3.2.2.3. Viscosity

For the binary sugar solutions a Cannon-Manning micro U tube size 450 was used in a large uncontrolled water bath with temperature measured to $\pm$ 0.01 °C. The viscometer was calibrated using an accurate 70% sucrose solution. This was to determine the viscometer constant, which is required to calculate the kinematic viscosity of a solution. For more viscous solutions like model honey solutions, a Brookfield DV-E viscometer with a 3.2 mm diameter spindle and a 9 mm inside diameter tube that was set in water jacket with temperature controlled to $\pm$ 0.02 °C was used. This was calibrated using Cannon N1000 calibration oil to within ±1% over the rotational speed range of the viscometer. All other viscosities were measured using Haake RotoVisco RV20 viscometer. Two sensor assemblies (NV for viscosities less than 10^5 mPa.s and MV1 for viscosities over 10^5 mPa.s) were used for different samples. Viscometer calibrations were performed prior to taking any measurements.

3.2.2.4. Refractive index

The refractive index of all the sample solutions was measured as a tool to validate the concentration. An Atago NAR-3T refractometer was used to determine the refractive index of the sugar solution. Calibration and measurements were carried out at 20 °C as outlined by the Atago NAR-3T refractometer operating conditions. A Grant bath GD120 water bath was attached to the refractometer to maintain the temperature of the sample at 20 ± 0.1 ºC during measurements. An average of three consecutive readings was used. The repeatability of refractive index measurements was $\pm$ 0.0005.

3.2.2.5. Water activity

Water activities of all sugar solutions were measured using a AquaLab 4TE (Decagon, USA) water activity meter. The water activity meter works using the principle of dew point temperature. The water activity meter calibration was checked frequently with standard solutions as purchased from the supplier with specified water activity value. Apart from

standard solutions, the water activity meter was also checked with deionized Milli-Q water with a precision of ± 0.0003 (water activity). The primary focus was to develop a sound technique to measure water activity to an accuracy of better than three decimal place. This was achieved by varying the measuring time and finding a minimum time of four hours for measurement. Finally, a continuous reading over longer periods of time was observed and a suitable reading was obtained from plotting the data over time. This method was developed following initial results as described in APPENDIX A.

3.2.2.6. Mutarotation studies

NMR studies

Glucose solutions of different mass fractions ($1\%, 10\%, 20\%, 28\%$) were freshly prepared in Milli-Q (deionised) water in LabServ 250 mL containers, sealed and mixed gently in order to prevent any air bubbles entrainment in the solution. To mark the delay time before acquisition, a stopwatch was started with the first drop of water touching the solid; depending on the concentration, it took approximately $5 - 40$ minutes for the sugar to dissolve. Once all the glucose was dissolved, 1 mL of it was transferred to an NMR tube. To provide a deuterium signal for spectrometer lock and a reference peak at 0 ppm, yet to avoid contamination of the sample, a capillary insert containing 4,4-dimethy l-4-silapentane-1-sulfonic acid (DSS) in deuterated water (D_2O) was placed in the tube. The data were collected using an Agilent 400 MR NMR spectrometer equipped with OneNMR probe. After transferring the sample into the spectrometer, the acquisition started immediately, and 200 ^1H spectra were recorded continuously with intervals of 90 s, which resulted in total experiment times of more than five hours. In each experiment, 16384 samples were recorded in single scans followed by excitation pulses with flip angle of $45°$; the temperature was set to 25 °C for all measurements though the solutions were prepared at ambient temperatures ranging between 20 and 25 °C.
To understand mutarotation kinetics of simple sugars in viscous sugar solutions, a new method was developed.

A sucrose solution of 80% mass fraction was prepared as per the standards of a binary solution as described in Section 3.2.1.1. Prior to the NMR measurement, a 22.7% glucose monohydrate solution was prepared by mixing the desired weight of glucose in Milli-Q water at ambient

temperature and timed since the first drop of water touched the sugar. Within $3-5$ minutes when the glucose was completely dissolved, this solution was poured into the sucrose solution and mixed gently preventing air bubbles but ensuring complete mixing. Then the new sample was placed inside the NMR tube. To estimate the relative mole fractions of the forms of glucose, their reference signals were fitted to each of the measured spectra in the least-squares sense. The estimated mole fractions is directly proportional to the corresponding concentrations. These were then fitted with the proposed three-parameter exponential model, separately for each form of glucose.

Optical rotation

A Perkin Elmer 341 polarimeter was used for the measurements of the optical rotation of the sugar solutions. The sodium D line monochromatic radiation ($\lambda = 589$ nm) with a 10 cm path length cell was used for the measurements. The light source was switched on at least one hour prior to measurement. Sugar solutions were freshly prepared with the time set at zero at the first drop of water touching the sugars in the sample container. Glucose samples $(1\%, 10\%, 30\%)$ were prepared at 20 ± 2 °C by dissolving α-D-glucose monohydrate in Milli-Q water. The usual time for mixing ranged from $5-40$ minutes based on solution concentration. Once a complete dissolution was attained at room temperature, it was poured into the cylindrical sample holder. Temperature control was achieved using a water bath attached to the sample holder. Optical rotation data was continually recorded every 100 s intervals for 10 hours. The measured values of optical rotation was plotted over time and fitted with an exponential decay with the initial time correction.

3.2.2.7. Crystallization

Some of the experiments below were designed and carried out under my supervision by final year undergraduate students. The list of students and their project titles can be found in APPENDIX F.

The focus of this investigation was to understand crystal growth rates and extent of crystallization using well-defined mixtures of water, fructose and glucose comparable to honey. Effect of additives like salt was also studies in these experiments. Physical properties like water

activity, refractive index were measured on these solutions over a continuous period of time. Microscopic analysis were also performed on some samples.

The first set of experiments were designed based on the fructose to glucose (F/G) ratio between the range 1.18– 1.5, with and without seeding of solution as detailed in Table 9. The total sugar concentration including any added seed crystals for all experiments was set at a mass fraction of 17%. All crystallization experiments were performed on invert sugars solutions with a water content of 17% by mass.

Table 9 Experiment design with parameters

Water content %	F/G ratio	S/G Ratio	Seeding %	Stirring
17.0	1.1800	0	1.0	No
17.0	1.2329	0	1.0	No
17.0	1.2329	0	1.0 & 0.1	No
17.0	1.5000	0	0.1	No
17.0	1.2329	0	0.1	Yes at 20 °C
17.0	11.290	0	0.0	Yes at 20 °C

The second set of experiments was designed focusing on different compositions in solutions mimicking simple to complex sugar systems. The effect of water, F/G ratio, sucrose to glucose (S/G) ratio, additives were the focus while measuring different parameters in this experiment set. The details can be found in Table 10.

Table 10 Experiment design with parameters

Components	Water Content %	F/G Ratio	S/G Ratio	Additives (NaCl, Manuka Honey) %	Seeding %
Glucose + water	30 - 50	1.18	-	-	-
Glucose + fructose + water	13 - 21	1.2329	-	-	-
Glucose + fructose + sucrose + water	13 - 21	1.2329	0.0411	-	-
Glucose + fructose + water	15	1.2329	-	-	-
Glucose + fructose + sucrose + NaCl + water	15	1.2329	0.0411	0, 0.02, 0.1, 0.5 & 1.03	-

In order to understand the effect of additives (salts and acids) on some of the physical properties in model honey solutions, a third set of experiments were designed and carried out. As a simplification, all of the minerals were replaced by sodium chloride. Similarly, when choosing an acid, malic acid was used as it is a weak organic acid present in natural honey (Tomasik, 2004), and also used as an acid present in model food systems (Chirife & Fontan, 1980). Hence,

two different experiments were designed. First, the salt concentrations were kept constant at 0% and 5% mass fraction of salt with varying moisture content of $80\%, 81.5\%, 83\%$ and 84.5% moisture content. For the second set of experiments, the moisture content was kept constant at 17% and the solids concentration was maintained at 83% inclusive of additives.The various compositions of the salts and acids in the model honey solution are listed below in Table 11.

Table 11 Model honey system composition variation with acid and salt. Total solids and water content is kept constant at 83% and 17% respectively.

Mass fractions in percentages of components in the model honey system					
Total Solids	Water content	Glucose	Fructose	Salt (NaCl)	Acid (Malic)
83.0	17.0	41.5	41.5	0.0	0.0
83.0	17.0	41.0	41.0	1.0	0.0
83.0	17.0	40.5	40.5	2.0	0.0
83.0	17.0	40.0	40.0	3.0	0.0
83.0	17.0	39.5	39.5	4.0	0.0
83.0	17.0	39.0	39.0	5.0	0.0
83.0	17.0	41.0	41.0	0.0	1.0
83.0	17.0	40.5	40.5	0.0	2.0
83.0	17.0	40.0	40.0	0.0	3.0
83.0	17.0	39.5	39.5	0.0	4.0
83.0	17.0	39.0	39.0	0.0	5.0
83.0	17.0	39.0	39.0	1.0	4.0
83.0	17.0	39.0	39.0	2.0	3.0
83.0	17.0	39.0	39.0	2.5	2.5
83.0	17.0	39.0	39.0	3.0	2.0
83.0	17.0	39.0	39.0	4.0	1.0

The crystallization experiments were designed purely as an indicator for further studies and hence, repeats were not performed. Discussions will be based on these experimental results only.

4 RESULTS & DISCUSSION

As detailed in Chapter 3, all experiments were performed and the results are presented in the form of tables and graphs with necessary discussion. Each physical property is discussed separately and some that overlap are addressed in detail.

4.1. Density

The literature data for density of simple and complex sugar solutions were analysed based on the calculated apparent density of the sugar (Equation 61) in solution and summarised below.

$$\rho_{sugar} = \frac{w_s}{(\frac{1}{\rho_{mix}} - \frac{w_w}{\rho_w})} \tag{61}$$

The apparent density of glucose in a glucose + water system is shown in Figure 21. The deviations or error in density measurements can be visualised through the apparent density calculations as can be seen when calculated using Equation (61). Though most of the density data available are widely accepted, the errors in these density measurements are magnified when we convert them to apparent density of the sugars.

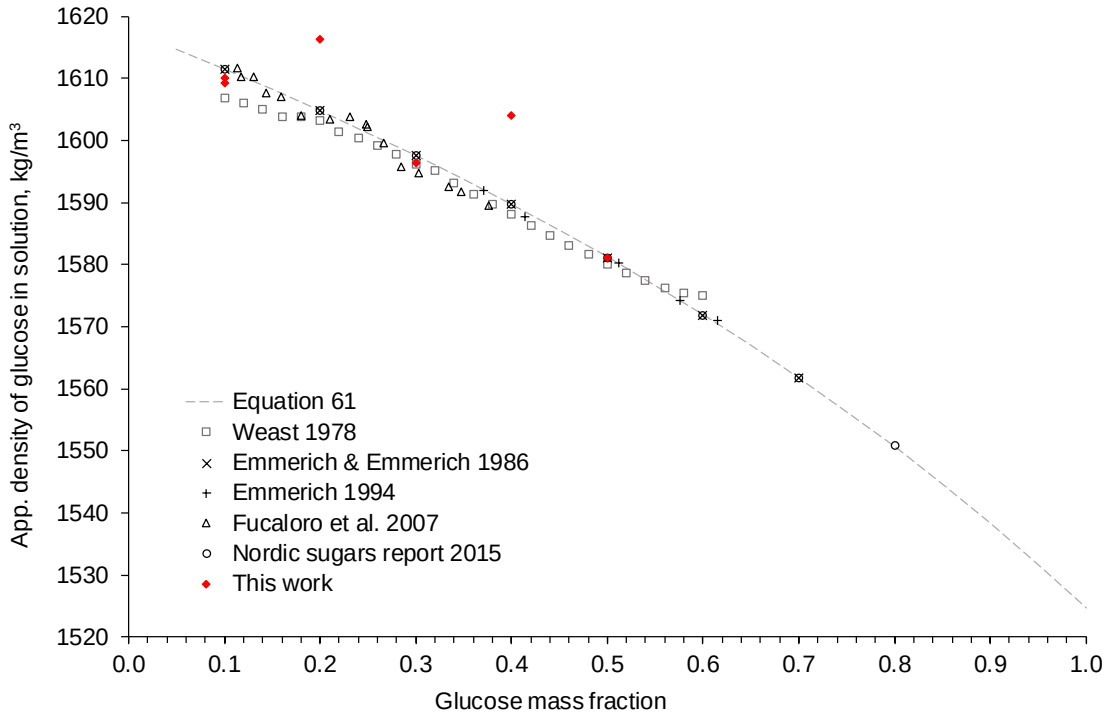


Figure 21 Apparent density of glucose in solution using Equation 61 in comparison with literature over a range of mass fraction at 20 °C.

Similarly, the apparent density data for a sucrose and fructose solution is shown in Figure 22 and Figure 23. Bettin et al. 1998 measured the density for glucose from 10% to 50% and 10 to 45 °C and for fructose from 10% to 70% and 10 to 50 °C and fitted equations through this data and further extrapolated the equations for the entire concentration range 0% to 100% and 10 to 80 °C. The calculated data was compared to literature data and the errors were 0.003 kg/m^3 and 0.011 kg/m^3 for glucose and fructose solutions respectively.

$$\begin{aligned}\rho_s = \rho_w + b_{01}.w + b_{02}.w^2 + b_{03}.w^3 + b_{04}.w^4 + b_{05}.w^5 + b_{06}.w^6 \\ + (b_{11}.w + b_{12}.w^2 + b_{13}.w^3 + b_{14}.w^4 + b_{15}.w^5).(t-20) \\ + (b_{21}.w + b_{22}.w^2 + b_{23}.w^3 + b_{24}.w^4).(t-20)^2 \\ + (b_{31}.w + b_{32}.w^2 + b_{33}.w^3).(t-20)^3 + (b_{41}.w + b_{42}.w^2).(t-20)^4\end{aligned} \quad (62)$$

The value of the coefficients, b_{ik} for Equation 62 are listed in Table 12 for glucose and Table 13 for fructose solutions.

Table 12 Coefficients of Equation 62 for glucose solutions

i	$k=1$	$k=2$	$k=3$	$k=4$	$k=5$	$k=6$
0	382.3089	122.8456	33.7382	−10.9724	15.7115	−17.0990
1	−0.55131	−0.01651	0.12055	0.06328	0.13662	
2	0.0075748	−0.000564	−0.0002244	−0.0024582		
3	$-4.3945e^{-5}$	$-1.6701e^{-5}$	$-6.554e^{-5}$			
4	0	0				

This principle can be extended to solutions with more than one solute or mixtures comprising of multiple components. Once the apparent density of the components in a mixture is known, then the density of the mixture can be calculated using Equation (1).

Table 13 Coefficients of Equation 62 for fructose solutions

i	$k=1$	$k=2$	$k=3$	$k=4$	$k=5$	$k=6$
0	389.9822	128.5980	41.2216	−20.4398	30.0894	−32.4503
1	−0.73991	−0.16423	0.17736	−0.06407	0.20928	
2	0.0077259	0.0013194	−0.0064035	0.0011841		
3	$-6.9542e^{-5}$	$-2.5768e^{-5}$	$7.7592e^{-5}$			
4	$-3.033e^{-7}$	$-1.919e^{-7}$				

The available data is shown as apparent density of glucose in solution in Figure 21. The Weast (1978) data showed considerable deviation of up to 5 kg/m^3 at low sugar mass fractions and a close to consistent difference to the fitted equation for the rest of the mass fractions. The last two data points shows a bigger deviation. The inconsistencies in this data maybe due to some kind of smoothening effect. While, the Fucaloro et al. (2007) has both positive and negative deviations from the fitted equation and this may be the experimental error. Data from Nordic

Sugar Report (2015), Emmerich and Emmerich (1986), Emmerich (1994) show good fit to the fitted equation.

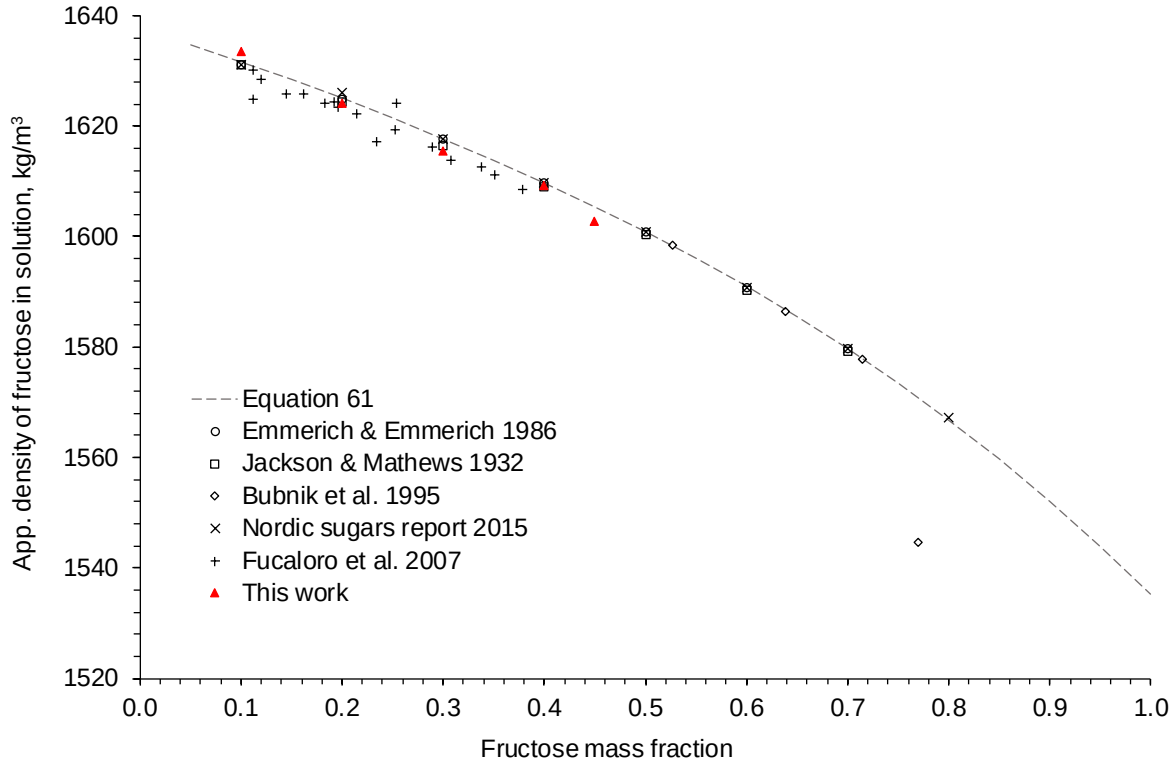


Figure 22 Apparent density of fructose in solution using Equation 61 in comparison with literature over a range of mass fraction at 20 °C.

Similarly, Figure 22 shows the apparent density of fructose in solution as compared to fitted Equation 61. Emmerich and Emmerich (1986), Jackson and Mathews (1932), Nordic Sugar Report (2015) data shows considerable agreement with the fitted equation. Bubník et al. (1995) data was a good fit except for the last data point at 0.8 mass fraction which might be a typographical error. Fucaloro et al. (2007) data deviations could be due to the reason that the measured densities are in the low sugar mass fractions.

It is found, and seen in the figures, that the apparent density of the sugars depends on the mass fraction of the sugar. In some cases there is data for the effect of temperature also. The relationships were fitted for the "best" data excluding the outliers and unreliable ones, and relationships were found with the quadratic form:

$$\rho_{sugar} = a + bw_s + cw_s^2 \tag{63}$$

For sucrose a data set was available from Barber (1965) between 0 and 75% mass fraction, and 0 to 30 °C. Least squares fitting yielded:

$$a = 1662.7 - 2.5025T + 0.0306T^2 \tag{64}$$

$$b = -57.953 + 2.2511T - 0.0417T^2 \tag{65}$$

$$c = -40 \tag{66}$$

Figure 23 shows the apparent density of sucrose in solution of different literature data in comparison with the fitted equation. The data of Emmerich (1994), Nordic Sugar Report (2015), Chenlo et al. (2002) fitted well with Equation (64). Weast (1978) data shows deviation of up to 2 kg/m^3 at the lower mass fraction and gets a better fit as the mass fraction of the sugar increases. The data of Fucaloro et al. (2007) showed high deviations and is discarded as it is not worth any further discussion.

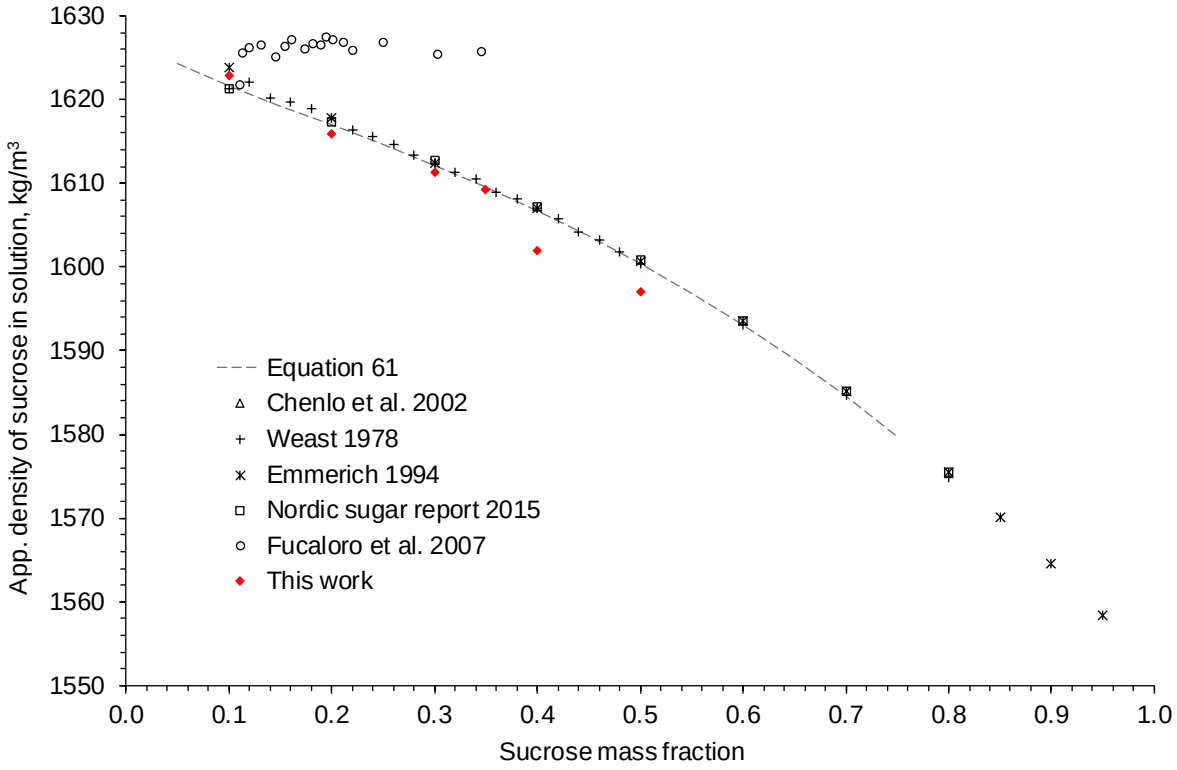


Figure 23 Apparent density of sucrose comparison with literature over a range of mass fraction at 20 ºC with Equation 61

Comesaña et al. (2003) measured the densities of mixtures of glucose + NaCl + water. The density data for the mixture was compared to the calculated density of the mixture using Equation (1). Here, the apparent density of glucose was calculated using Equation (61) based

on glucose mass fraction while apparent density of NaCl was calculated based on the mass fraction of water. Equation (1) was used to calculate the apparent density of NaCl, and it was found to depend on the mass fraction of water rather than the mass fraction of sugar or NaCl, as shown in Figure 24. Rather than eliminate outlying data to obtain a least squares fit, a line was fitted by eye as $\rho_{NaCl} = 1150w_w + 1980$. The deviation at low solids content is acceptable due to the high errors in the calculation of apparent density, and also knowing that the results will be applied in concentrated solutions.

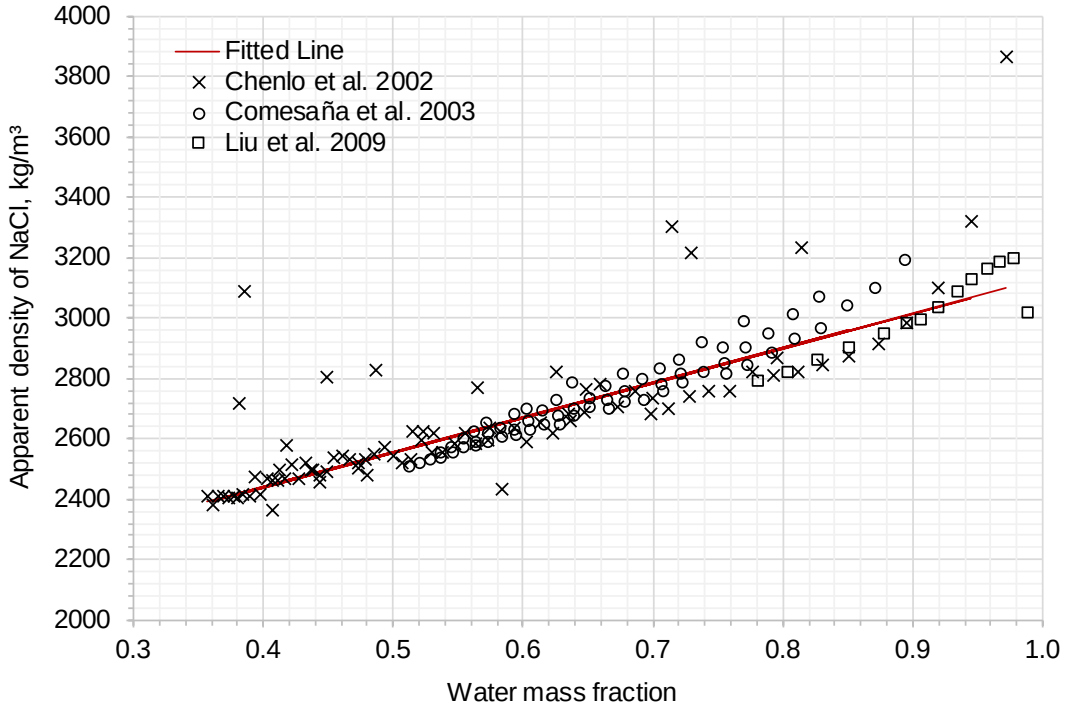


Figure 24 The apparent density of NaCl on the basis of water mass fraction. An equation is fitted by eye as shown.

The calculated density data of the mixture had good agreement to the experimental data as can be seen in Figure 25.The maximum error calculated was 1.6 kg/m^3. The dependence on water mass fraction have already been seen in Equation (21) for viscosity and Ross's approach for water activity of multi-component system described as Equation (41) in Section 2.3.5.

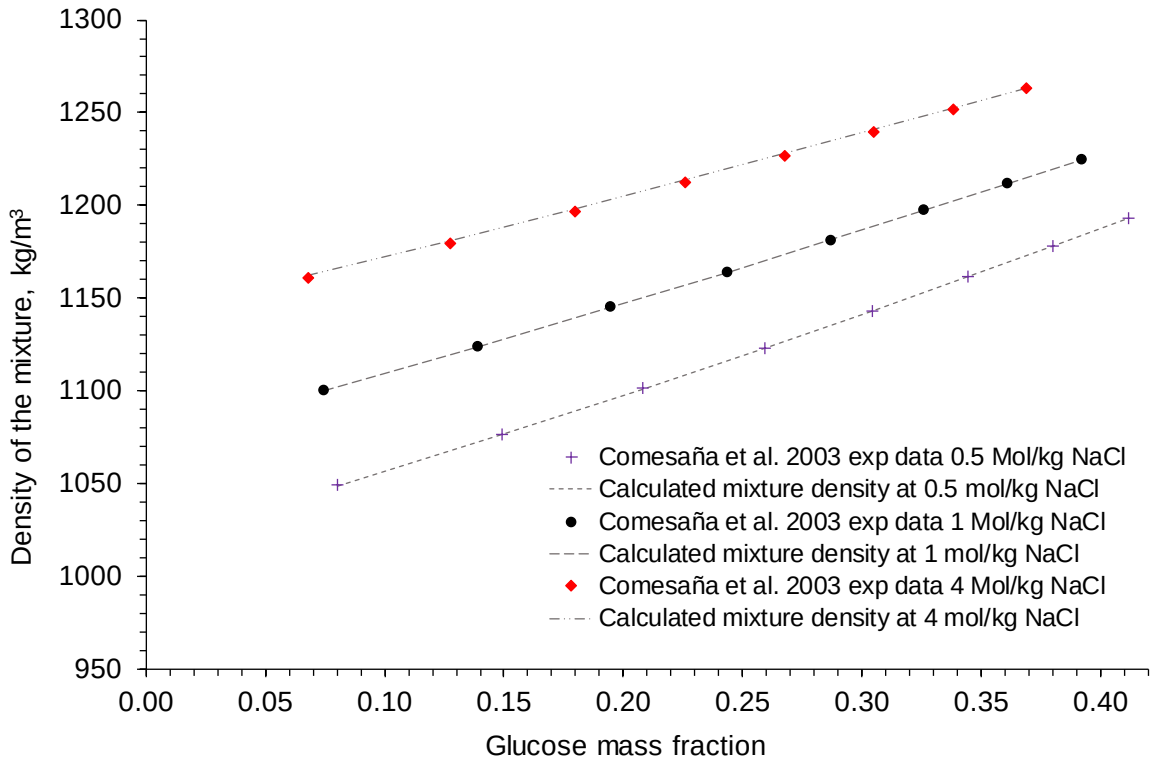


Figure 25 Comparison of measured density of glucose + NaCl + water mixture and the calculated density of mixture using Equation (1) at 20 ºC (Comesaña et al., 2003).

The deviations in the measured and the calculated values of density at 25 ºC of the sucrose + NaCl + water mixture of two different data sets were analysed and compared in Figure 26. The data of Banipal et al. (2002) showed a better agreement with the calculated values of the mixture with a maximum difference of 2.7 kg/m^3 in comparison to 5.7 kg/m^3 in the Chenlo et al. (2002) data. These difference between data sets indicates the errors involved in the measurement.

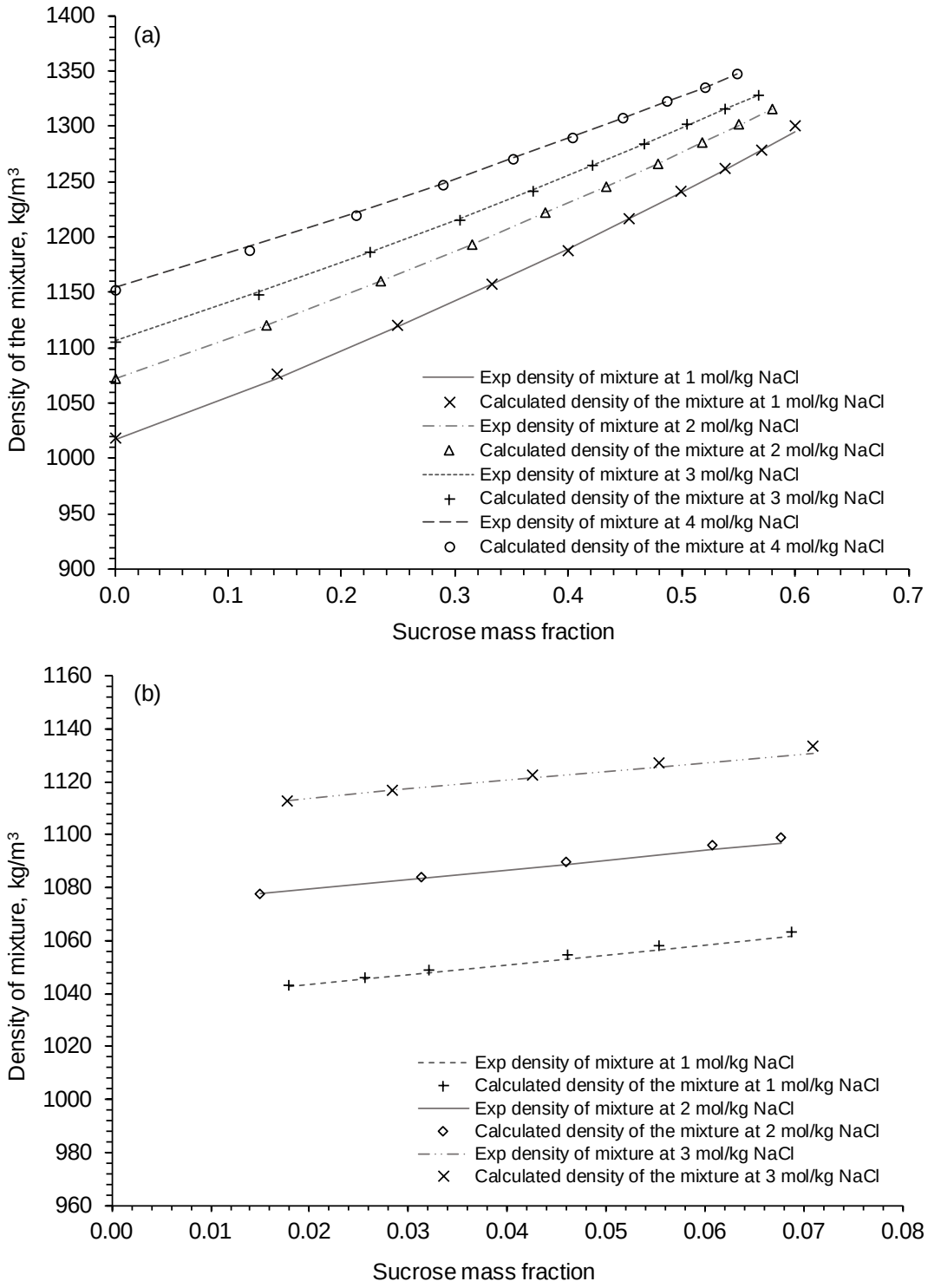


Figure 26 Comparison of measured density of sucrose + NaCl + water mixture and the calculated density of mixture using Equation (1) at 25 ºC. (a) Chenlo et al. (2002);(b) Banipal et al. (2002)

What happens when extending a similar approach to sugar mixtures? Figure 27 shows the relative errors of the experimental density values to the literature data of the same sugar mixture at 20 ºC. The relative errors show an sharp increase above sugar mass fraction of 0.5. The

density of invert sugar mixtures at 20 ºC was calculated using Bettin's equation (59) for the apparent density of glucose and fructose together with Equation (1). The comparison to the four literature data sets showed a maximum discrepancy of 1.1 kg/m^3 (0.08%). At high concentrations the experimental data were greater than the values calculated by the equation. This shows the reliability of the calculated values based on the equation.

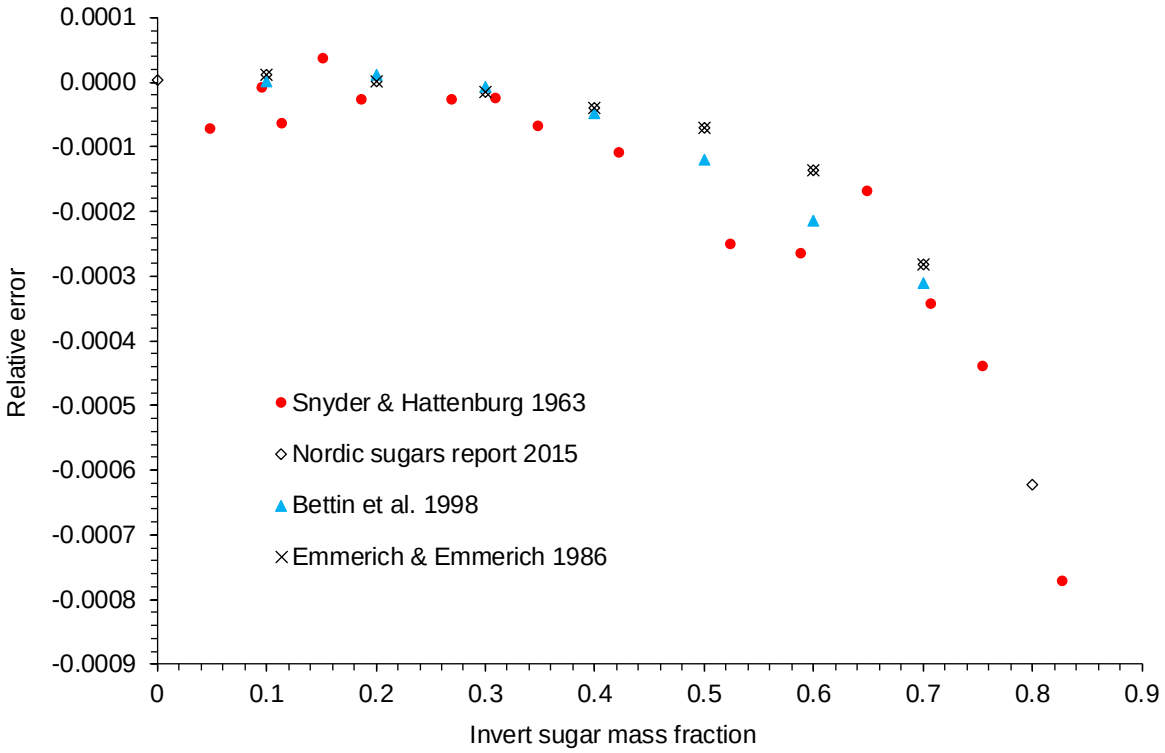


Figure 27 Relative error (experimental-calculated) to the calculated density values of the invert sugar mixtures using Equation (1) and literature data of invert sugar mixtures at 20 ºC

Table 14 Absolute and relative error of densities of model honey solutions at 20 ºC

Water mass fraction	Calculated density of the mixture (Equation 1)	Experimental density of the mixture	Difference	Relative difference,
	kg/m^3	kg/m^3	kg/m^3	%
0.2099	1421.36	1423.11	-1.7	-0.12
0.1900	1436.17	1438.36	-2.2	-0.15
0.1701	1451.21	1452.15	-0.9	-0.06
0.1502	1466.61	1462.53	4.1	0.28
0.1310	1481.69	1480.89	0.8	0.05

Equation (1) is further used to predict the density of model honey systems with extremely high sugar concentrations. Results of the experimental values from the current work and calculated

values are plotted on Figure 28 as a function of water mass fraction. The model honey mixture comprises of glucose, fructose, sucrose and NaCl in honey ratios given in Table 8. The measured and calculated densities of the model honey solutions had a maximum difference of 4.07 kg/m^3, which is 0.28% error in measurement. The error does not seem to be consistent so it is likely to be mostly from experimental error. Table 14 shows the errors between the measured and the calculated densities of the model honey solution.

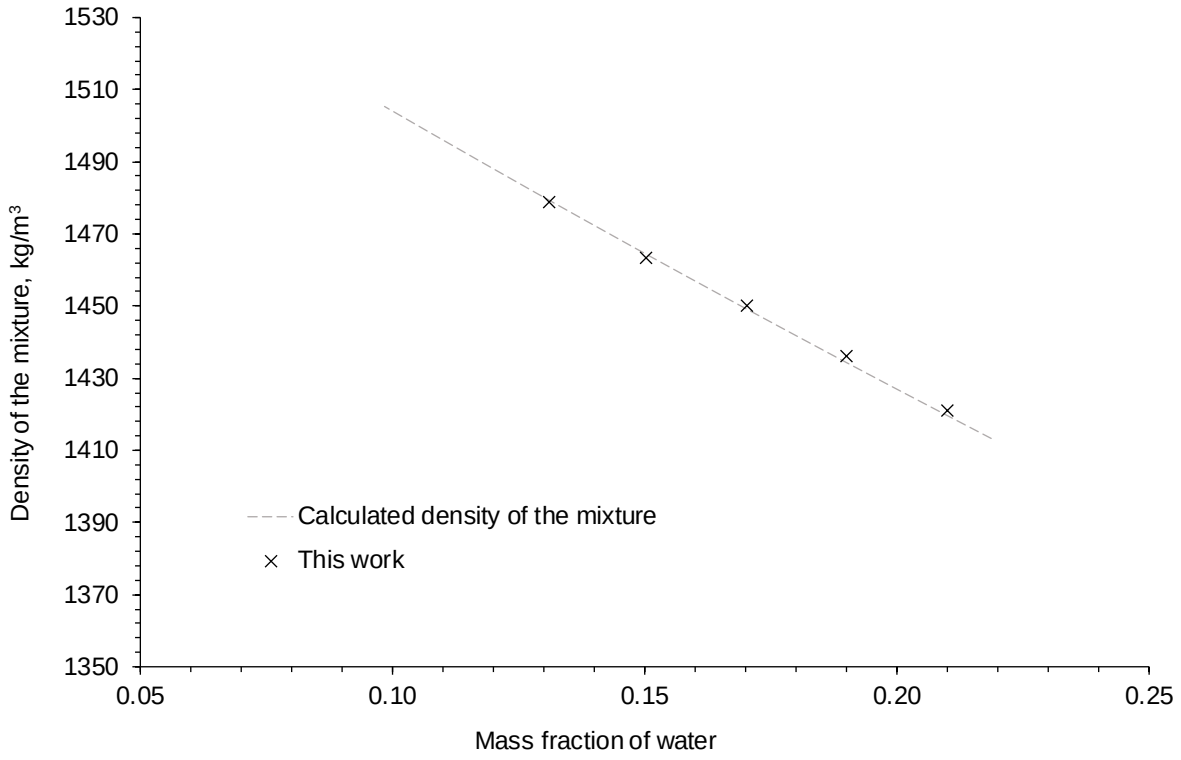


Figure 28 Measured and calculated (Equation 1) density of model honey solutions at 20 ºC over a range of water mass fractions.

The experimental values of densities for mixtures with sugars + salts + acids were in good agreement with the calculated values. Thus, the values calculated for the model honey solutions on the basis of mass fractions of solids and apparent densities has proved to be a dependable method and a good extension to mixtures involving electrolytes and non-electrolytes.

4.2. Viscosity

The bulk viscosities, η, of binary solutions of glucose, fructose and sucrose were obtained from Weast (1978) and equations were fitted in the form of Equation 67 recommended by Morison and Hartel (2007) to obtain constants.

$$\eta_i(T) = \eta_w(T) e^{\sum_i a_i \frac{w_i}{w_w} + b_i \left(\frac{w_i}{w_w}\right)^2} \tag{67}$$

Here T is the temperature, w is mass fraction, a and b are constants for a binary solution, subscript i refers to a single component i and subscript w refers to water.

Figure 29 shows the available literature data on the viscosity of glucose solution at 20 ºC at a range of glucose/water mass ratio and the experimental data showed a maximum deviation is 4.3×10^{-4} Pa·s. Most literature data fitted well with the proposed Equation (67) while the experimental data showed a maximum deviation of 7.0×10^{-4} Pa·s at the highest mass fraction. The viscosity data are plotted as logarithm of viscosity versus w_{sugar}/w_{water} showing that the relationship is almost linear at low to medium concentrations. This convention was used for all viscosity data across all sugar concentrations.

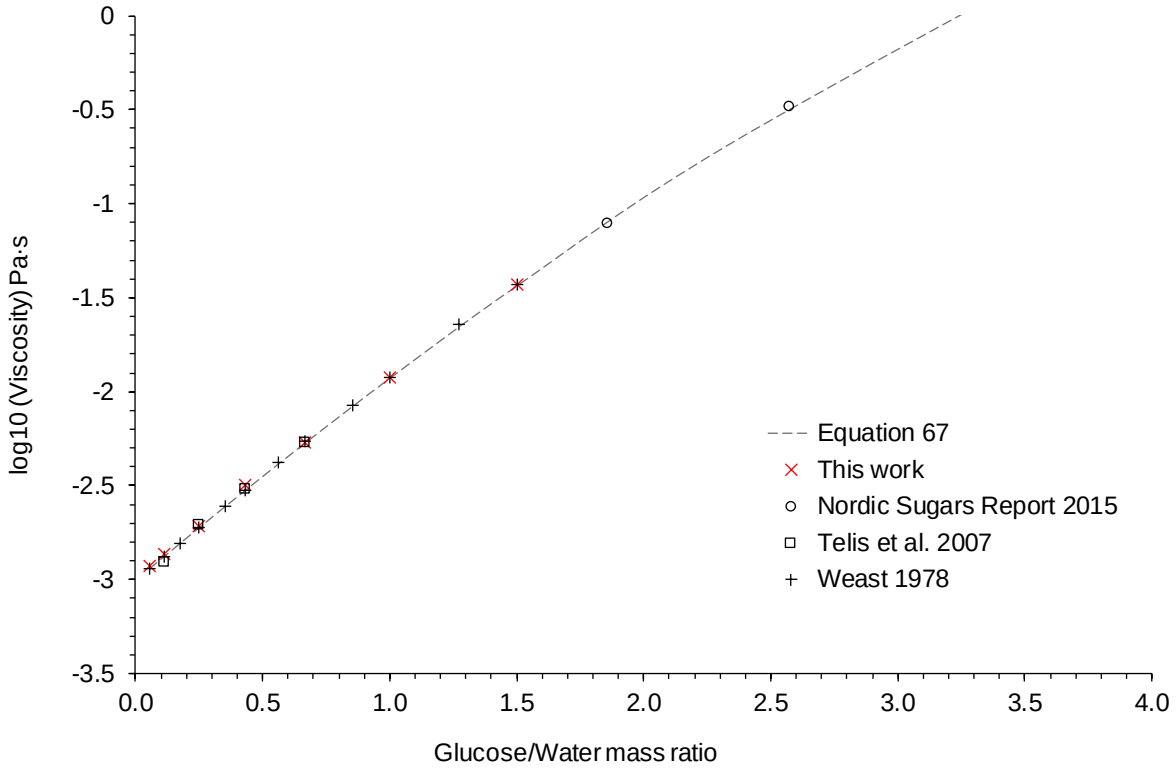


Figure 29 Bulk viscosity data for glucose solution available in the literature compared to the fitted equation at 20 ºC

In contrast, Figure 30 shows that for fructose solutions most literature data fits well with the equation, however, the data from Nordic Sugar Report (2015) showed considerable deviations from the equation over their measured mass ratios. Data from Telis et al. (2007), Weast (1978) and Rampp et al. (2000) all showed very good fit to Equation 67. This again emphasises the effectiveness of the proposed equation.

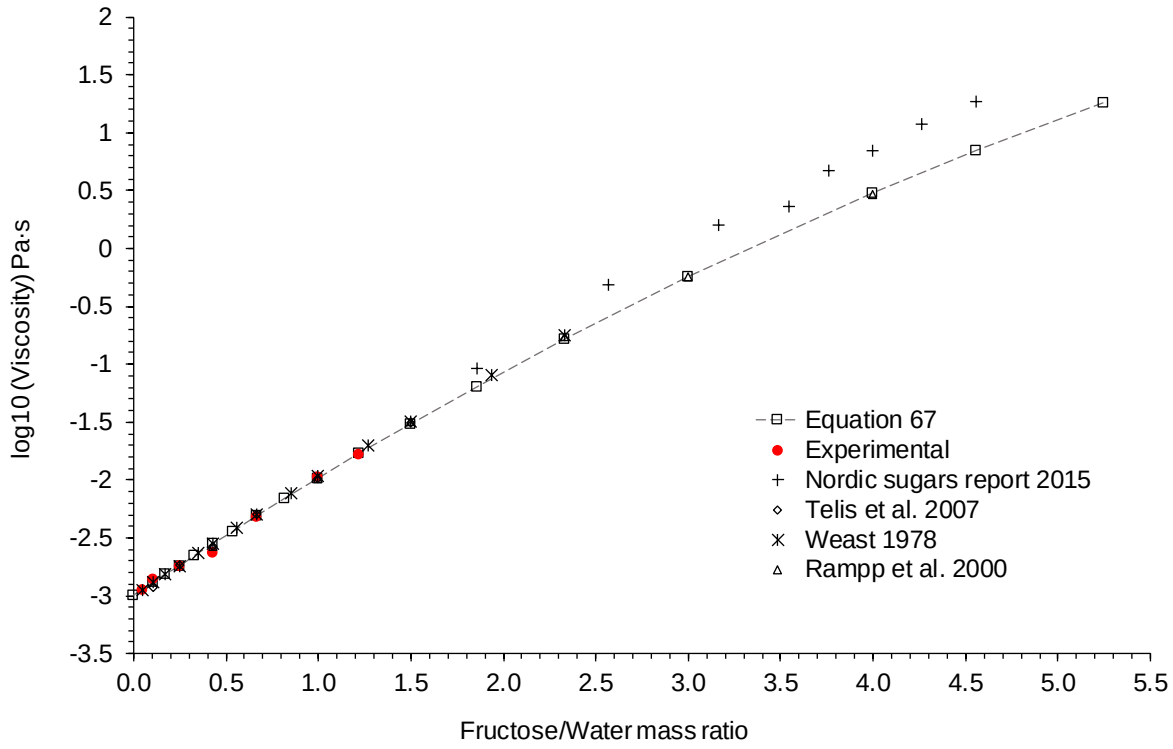


Figure 30 Bulk viscosity data for fructose solution available in the literature compared to the fitted equation at 20 ºC

For binary sucrose solutions, the viscosities from literature fitted well with the proposed Equation (64). Quintas et al. (2006) data showed deviations for the mass ratio between 2.5 to 3.5, however, the data fit reasonably well for ratios over 4. The experimental viscosities had a maximum deviation of 2.8×10^{-4} Pa·s

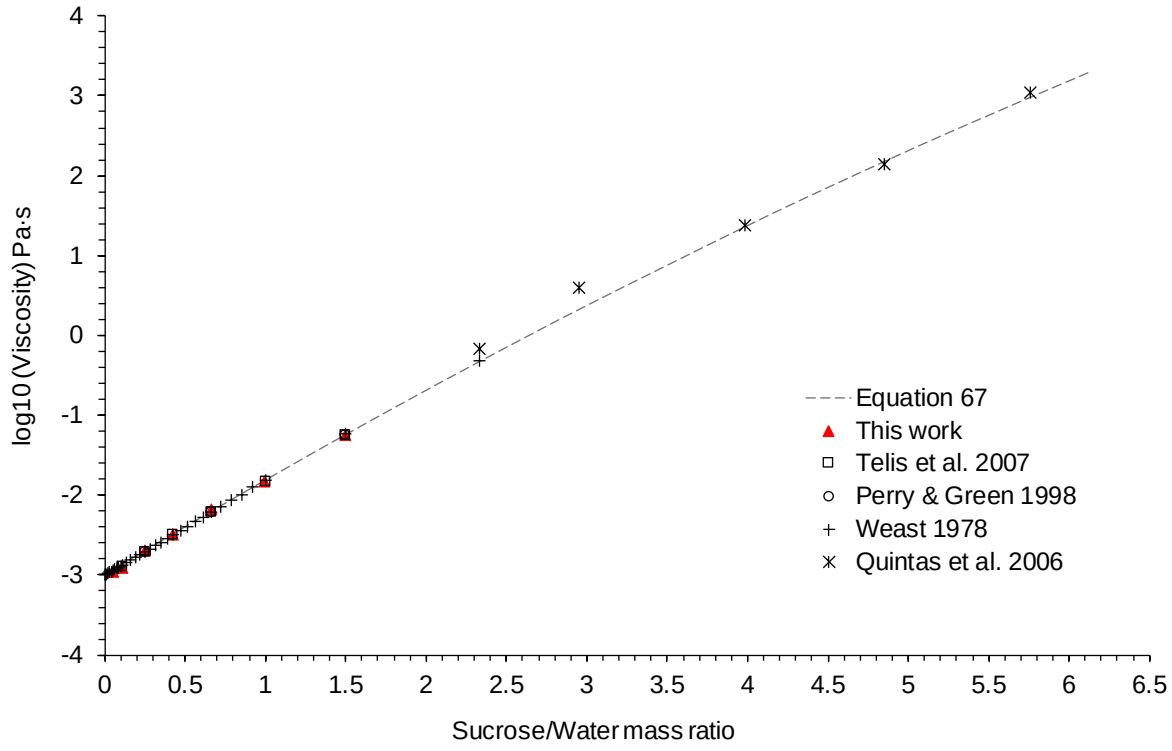


Figure 31 Bulk viscosity data for sucrose solution available in the literature compared to the fitted equation at 20 ºC

Viscosity data was obtained for model honey solutions with sugar concentrations from 68% to 84%. Similar to binary solutions, when plotted as logarithm of viscosity versus w_{sugar}/w_{water} a close to linear relationship is obtained at low to medium concentrations. A downward curvature is noticeable beyond about 60% sugar (sugar:water ratio > 1.5).

For mixed sugar solutions with total concentrations greater than about 60% it was found necessary to add an additional term to Equation (68) for the total mass fraction of sugars. The correction could not be applied to the individual sugars as the total concentration of sugar was beyond any possible binary solution concentration.

$$\eta_i(T) = \eta_w(T) e^{\sum_i \left(a_i \frac{w_i}{w_w} + b_i \left(\frac{w_i}{w_w} \right)^2 \right) + a_T \frac{w_T}{w_w} + b_T \left(\frac{w_T}{w_w} \right)^2} \quad (68)$$

It was found that values for $a_T = 0$ and $b_T = -0.045$ gave an adequate fit as shown in Figure 32 and Figure 33. While the experimental values fitted well with maximum deviation of 3.7 Pa·s using Equation (68) for invert sugar mixtures up to 86% mass fraction, the Nordic Sugar Report (2015) data showed highest deviations of up to 42 Pa·s from equation.

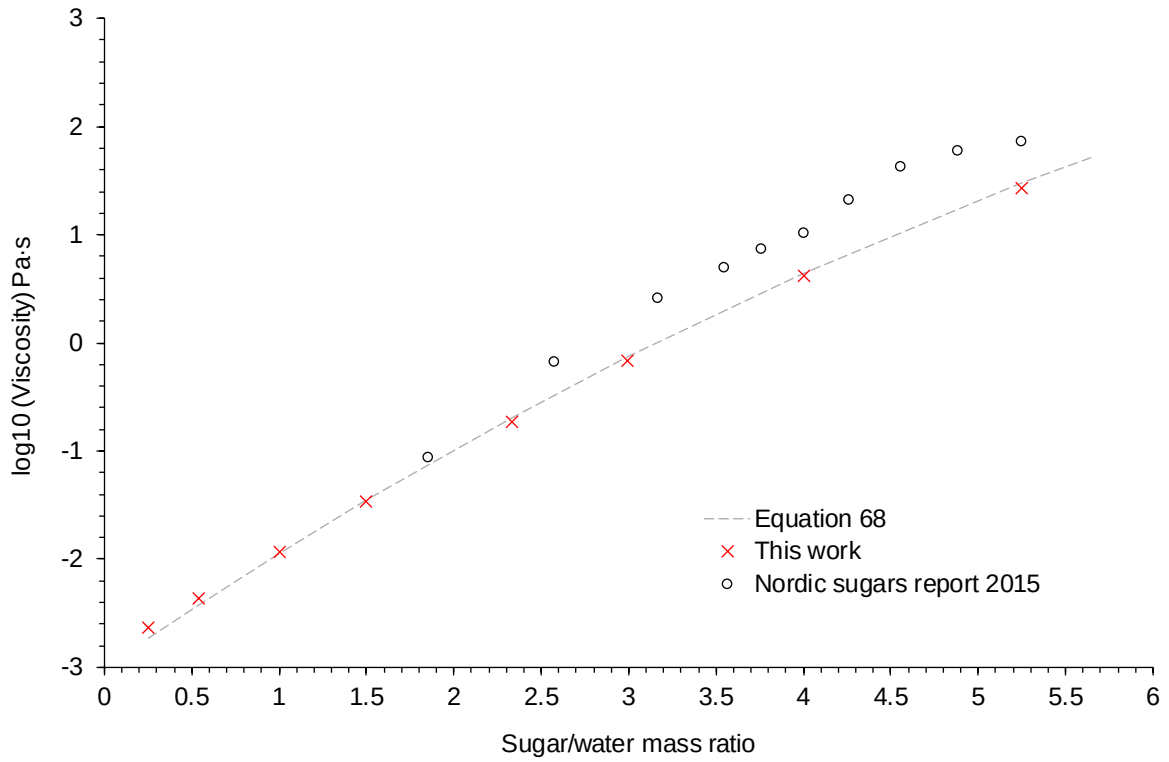


Figure 32 Viscosity data for invert sugar solution available in the literature compared to Equation 68 at 20 ºC

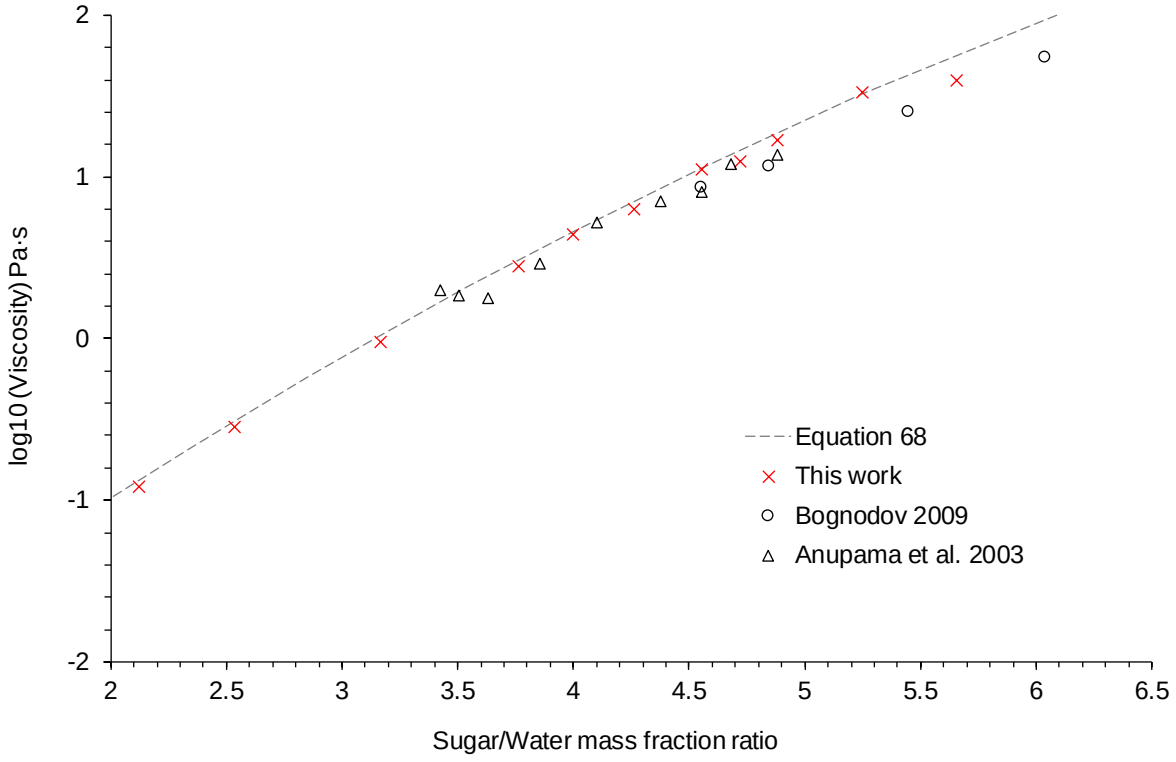
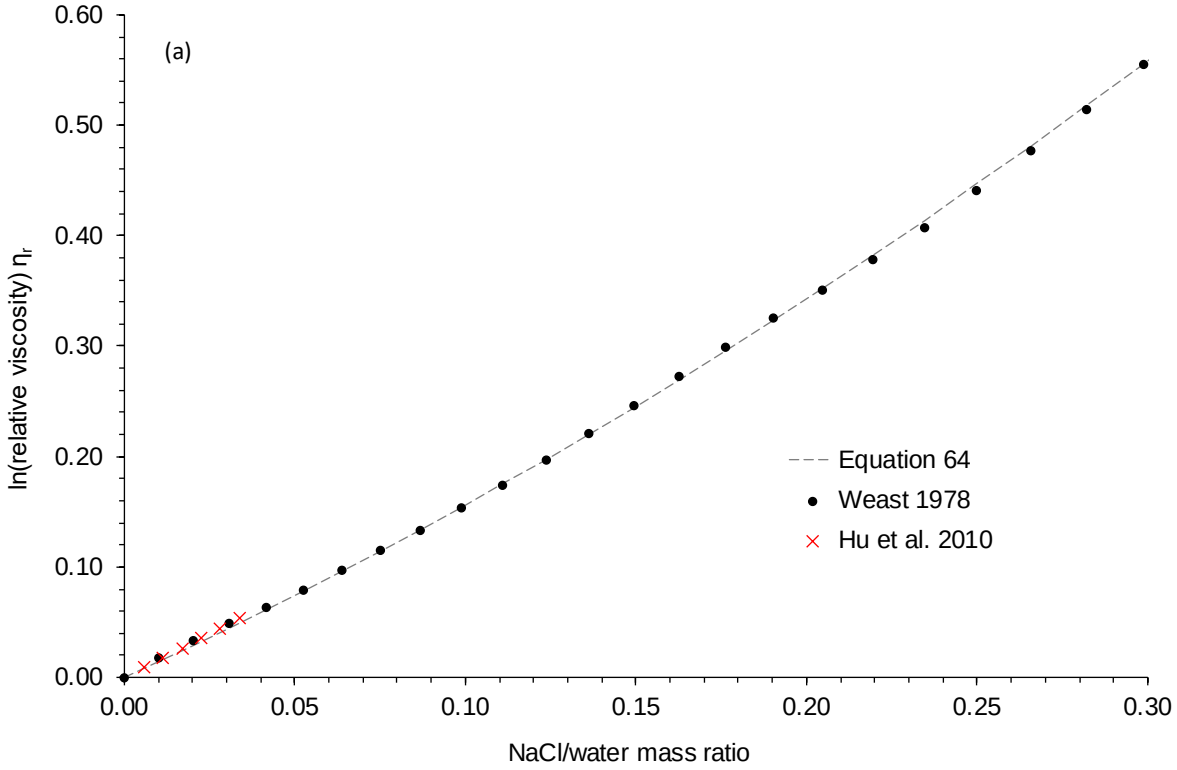


Figure 33 Viscosity relationship for concentrated sugar mixtures of glucose + fructose + sucrose (model honey) in honey ratio as per Table 8 at 20 °C.

Table 15 Constants for Equation (64) and (65) at 20 ºC

	a	b
Sucrose	2.79904	0.0
Fructose	2.44830	-0.1110
Glucose	2.60971	-0.1400
Malic acid	2.10160	-0.7875
NaCl	1.40900	1.5120

The coefficients a, b of the fitted values for Equation (64) and Equation (65) is tabulated in Table 15 and values for $a_T = 0$ and $b_T = -0.045$ as mentioned earlier. The fitting parameters for NaCl was obtained using the measured experimental viscosity by Weast (1978) and that of malic acid was obtained from experimental data of Chmielewska and Bald (2008). Figure 34 shows that Equation 64 can be extended to electrolyte solutions.



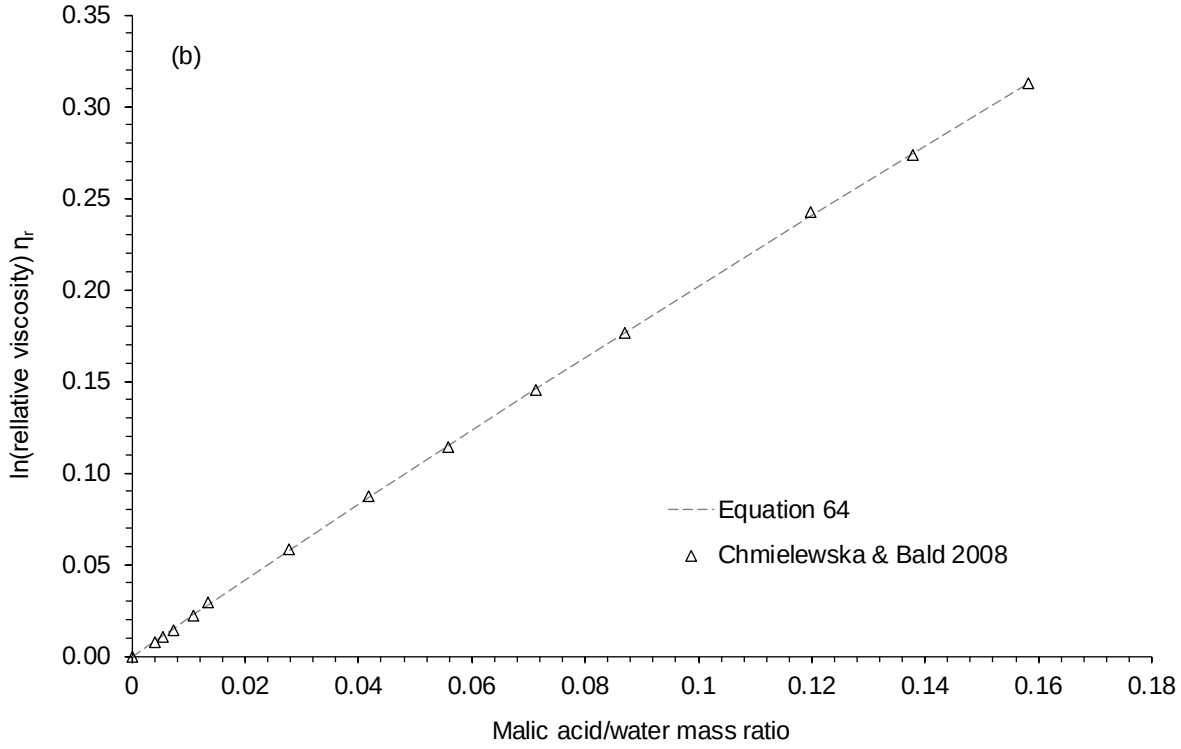


Figure 34 Application of Equation 64 to electrolyte solutions. (a) Malic acid (Chmielewska & Bald, 2008); (b) NaCl (Weast, 1978), (Hu, Zhang, et al., 2010)

Hence, after obtaining all the fitting parameters, a model systems was considered with glucose, fructose, sucrose, sodium chloride and malic acid. The viscosity for such a system is predicted in Figure 35. The variation in viscosity in terms of temperature can also be predicted using the equation.

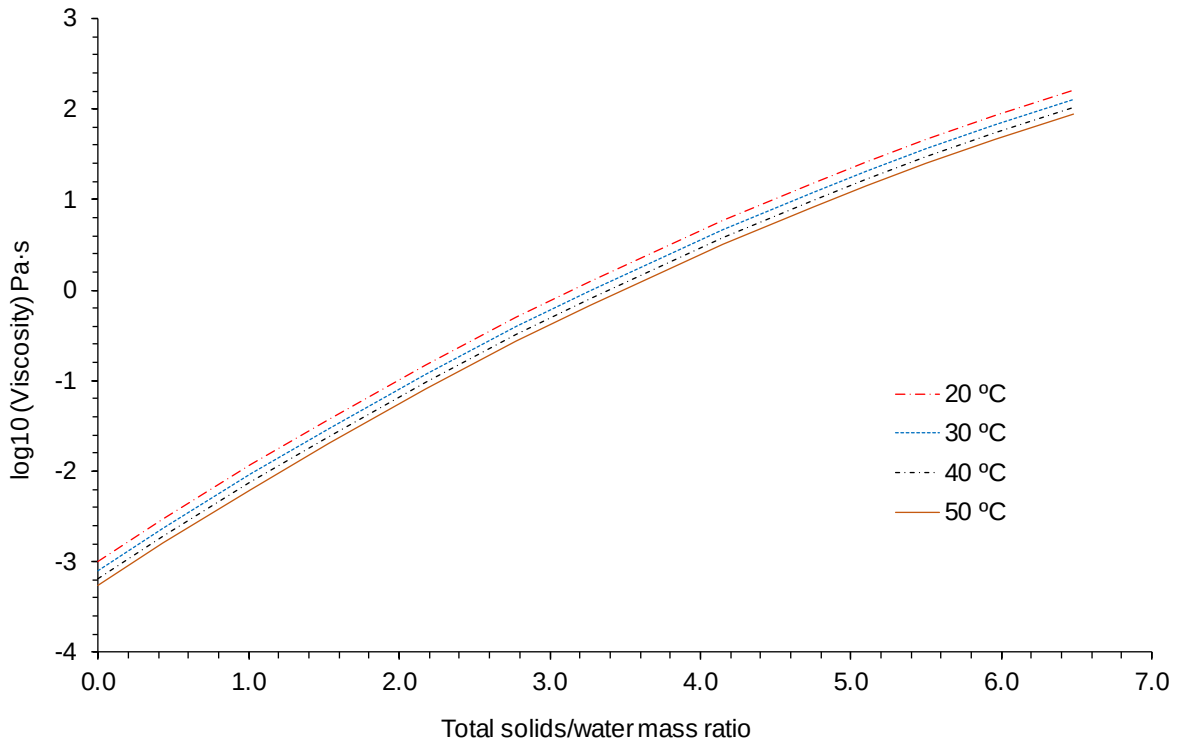


Figure 35 Viscosity prediction for a model honey system with glucose + fructose + sucrose + NaCl + malic acid (Table 15) at different mass fractions and temperatures.

Thus, the viscosities calculated using the proposed equation on the basis of sugars/water ratios at the temperature of the water viscosity is well suited for model honey systems. Further, the extension of the equation to electrolyte solutions has further added credibility to the claim.

4.3. Electrical Conductivity

4.3.1. Model Development

A mixture of glucose, fructose, sucrose, salts represented by sodium chloride and acid represented by malic acid is considered. The acid (designated as MH) and each of the sugars, glucose (GH), fructose (FH) and sucrose (SH) was considered to dissociate in water into a sugar anion, e.g., G^- and $H^+$with a dissociation constant K_a:

$$K_{aG} = \frac{[G^-][H^+]}{[GH]} \tag{69}$$

$$K_{aF} = \frac{[F^-][H^+]}{[FH]} \tag{70}$$

$$K_{aS} = \frac{[S^-][H^+]}{[SH]} \tag{71}$$

$$K_{aM1} = \frac{[MH^-][H^+]}{[MH_2]} \tag{72}$$

$$K_{aM2} = \frac{[M^{2-}][H^+]}{[MH^-]} \tag{73}$$

With the sugars and acid species adding to the total molar concentration of each

$$[GH] + [G^-] = C_G \tag{74}$$

$$[FH] + [F^-] = C_F \tag{75}$$

$$[SH] + [S^-] = C_S \tag{76}$$

$$[MH_2] + [MH^-] + [M^{2-}] = C_M \tag{77}$$

Also there is water dissociation which is represented as

$$[H^+][OH^-] = K_w \tag{78}$$

For neutrality in the model honey solution

$$[H^+] + [Na^+] = [G^-] + [F^-] + [S^-] + [M^-] + [OH^-] + [Cl^-] \tag{79}$$

Many values are given for the dissociation constants of sugars. Here the values used for K_a were: sucrose 1.35×10^{-13} mol L^{-1} (Singh et al., 2007), glucose 6.6×10^{-13} mol L^{-1}, and fructose 8.8×10^{-13} mol L^{-1} (Woolley et al., 1972). Malic acid has p$K_a$values of 3.46 and 5.1 for the first and second dissociation (Martell, 1976).

Equations (69) to (79) can be solved for the concentrations of ions and undissociated species using Newton's method. Once the ion concentrations were known, it was assumed that the electrical conductivity could be determined from Equation (80). It is acknowledged that an additional term could be added for high concentrations, but in all the work done, the ion concentrations were low.

$$\kappa = \Sigma_i C_i \lambda_i \tag{80}$$

where C_i is molar concentration of ions, and λ_i is the ionic conductivity, or using

$$\lambda_i = F z_i u_i \tag{81}$$

we get

$$\kappa = F \Sigma_i C_i z_i u_i \tag{82}$$

where F is Faraday's constant, z_i is the ion charge and u_i is the ion mobility. Ion mobility inversely related to viscosity, η using the Walden equation (Equation 9).

For binary aqueous electrolyte solutions, as the molar salt concentration, C, increases the calculated conductivity decreases and is often described by Kohlrausch's Law. It is clear here to use the molar concentrations as the amount of charge is directly related to the molar concentration.

$$\begin{gathered} \Lambda_m = \Lambda_m^0 - K C^{0.5} \\ \sigma = C \Lambda_m \end{gathered} \tag{83}$$

where Λ_m and Λ_m^0 is the molar conductivity of the solution and at infinite dilution respectively, and K is a constant. Using this as a basis, with the inverse viscosity relation from the Walden equation, and with data from Chambers et al. (1956) at 25 °C the molar conductivity of NaCl is given at temperature T as

$$\Lambda_{M,NaCl} = \left(114.6 - 28.6 C_{\mathrm{NaCl}}^{0.5}\right) \frac{\eta_w(25)}{\eta_w(T)} \tag{84}$$

Here molar conductivity is in units of mS.L.cm^{-1}mol^{-1} and concentration is in mol L^{-1}. For KCl the equation is:

$$\Lambda_{M,KCl} = (144.3 - 32.6 C_{KCl}^{0.3}) \frac{\eta_w(25)}{\eta_w(T)} \tag{85}$$

Conductivity data for binary aqueous solutions of NaCl and KCl are available from Shreiner and Pratt (2004) and Weast (1978). van Rysselberghe and Nutting (1934) showed that for solutions of KCl + NaCl, cumulative addition of NaCl and KCl conductivities on molar basis

was satisfactory, if the component (binary) conductivities are calculated at the total salt concentration.

$$\sigma_{mix} = \Lambda_{m,mix} \sum C_i = \sum (C_i . \Lambda_{m,i}) \tag{86}$$

The equation can be merged to isolate the effect of viscosity while combining the effect of concentrations of mixed solutes. Combining various Equations in a more general form for a vector of concentrations in a mixed solution, **C**, we can write:

$$\sigma = \frac{f(\mathbf{C})}{\eta} \tag{87}$$

It is proposed to obtain data from dilute solutions with a low viscosity, η^o, to obtain estimates of $f(C)$ for each of the components in a solution and combined using an equation of the form of Equation (86). In food systems the exact composition is often unknown so it is intended to fit the data using an effective concentration of all salts lumped together as NaCl.

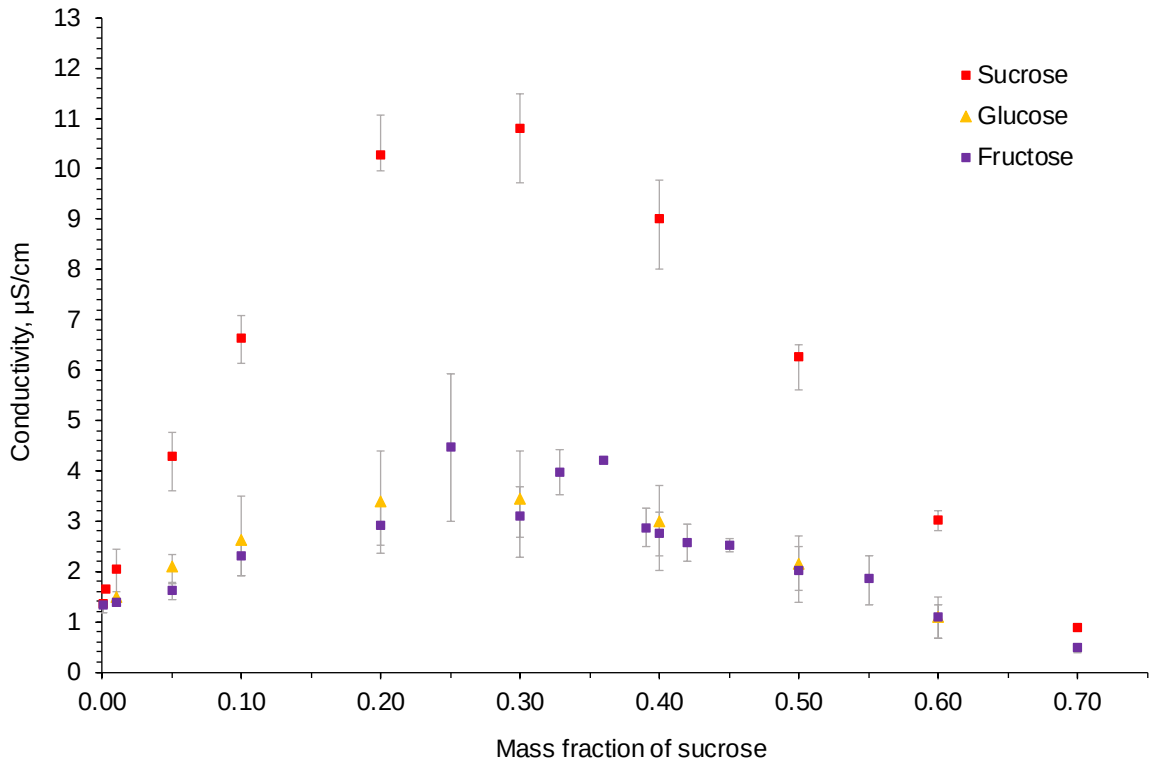


Figure 36 Experimental electrical conductivities of binary sugars solutions at 20 ºC with maxima and minima at each measured mass fraction to show the variations in the salt contents in the sugars and also measurement errors.

Figure 36 shows the conductivities of binary sugar solutions represented as a function of mass fraction at 20 ºC. Binary sugar solutions show a curvilinear behaviour with conductivity

reaching a maxima at 30% solids a marked decline in conductivity as the viscosity rises further. This has been well studied and is believed to be due to sugar molecules impeding the path of the ions. The conductivities of the binary sugar solutions were measured at different times of this research work over a period of three years and using different batches of sugar. The change in conductivities of the solutions in the entire measurement range is represented as the maxima and minima, over the mean values. These variations can be attributed to the salt present in the sugars during manufacturing process. A good representation of the variation of minerals in individual sugars. A visual estimate of roughly 3 times the conductivity of mono-saccharide minerals in a disaccharide solution can be observed. The conductivity at the final mass fraction is variable and this could be the effect on conductivity or the sensitivity of the conductivity cell. Disaccharide has more minerals and its conductivity at the same mass fraction is nearly twice the conductivity of single sugar (glucose and fructose about 0.5; sucrose about 0.9).

The conductivity of each binary solution was compared with model results, and hence an estimate could be made of the dry basis salt content of each sugar. For example, the conductivity of glucose solutions could be matched reasonably well by adding a dry basis NaCl mass fraction in glucose of 9.84×10^{-6}. The corresponding values for sucrose and fructose were 3.74×10^{-5} and 8.5×10^{-6}. These values are expected to vary between batches of sugars. These mass fractions are very small, but have a significant effect on the conductivity of the sugar solutions. Alternatively, it can be seen that conductivity can be used to determine the salt content of sugars.

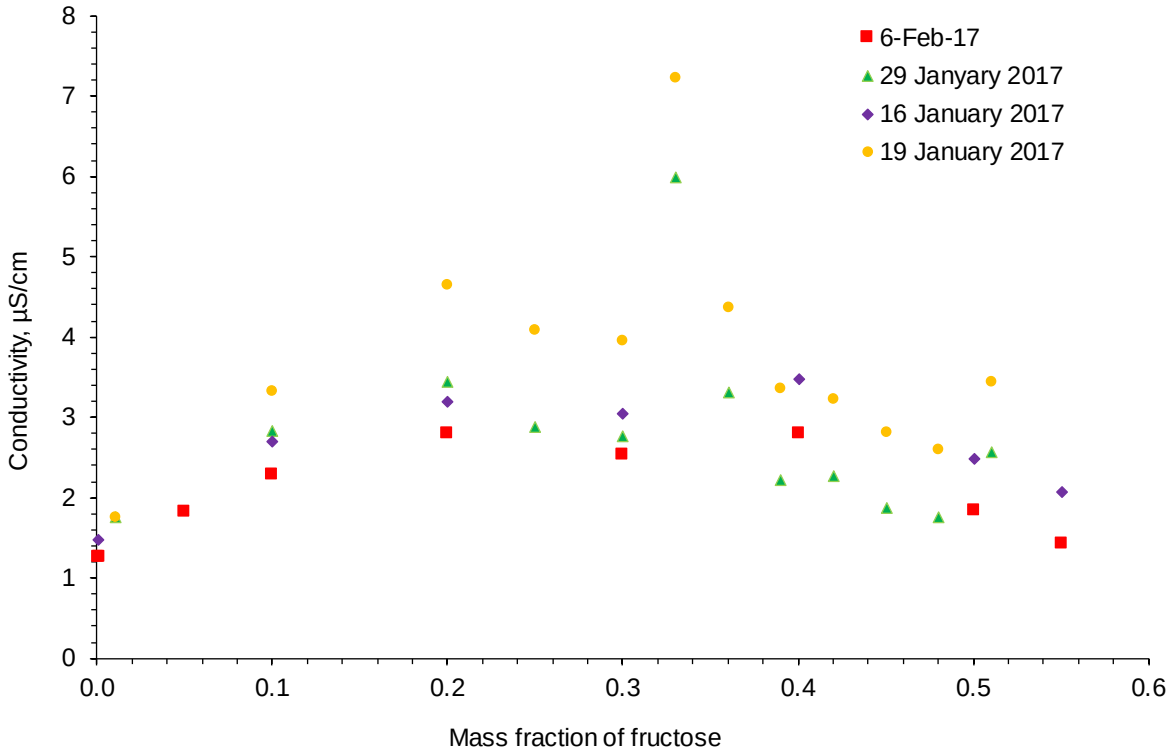


Figure 37 Conductivity of fructose + water solution over a range of mass fraction and, different days and batches at 20 ºC

The variations in the conductivities of fructose solution is erratic over the mass fraction range 0.2 to 0.4. Repeated measurements have shown conductivity variations as in Figure 37 that needed to be comprehended. A possible reason for this variation in this mass fraction range is mutarotation. Pigman and Isbell (1968) did mention that electrical conductivity measurements could not be used to understand mutarotation kinetics as it does not allow accurate calculations of reaction constant. Wang et al. (2013) showed that viscosity measurements could be used to measure mutarotation of anhydrous fructose. Though 6 to 12 hours of time for any reaction was provided, which is well above the time of 4 hours reported in literature (Perles & Volpe, 2005) for mutarotation in sugar solutions, some kind of reaction still happens over this period. An effort was made to understand this phenomena in fructose solution but, no cause was determined and it was decided not to pursue it further.

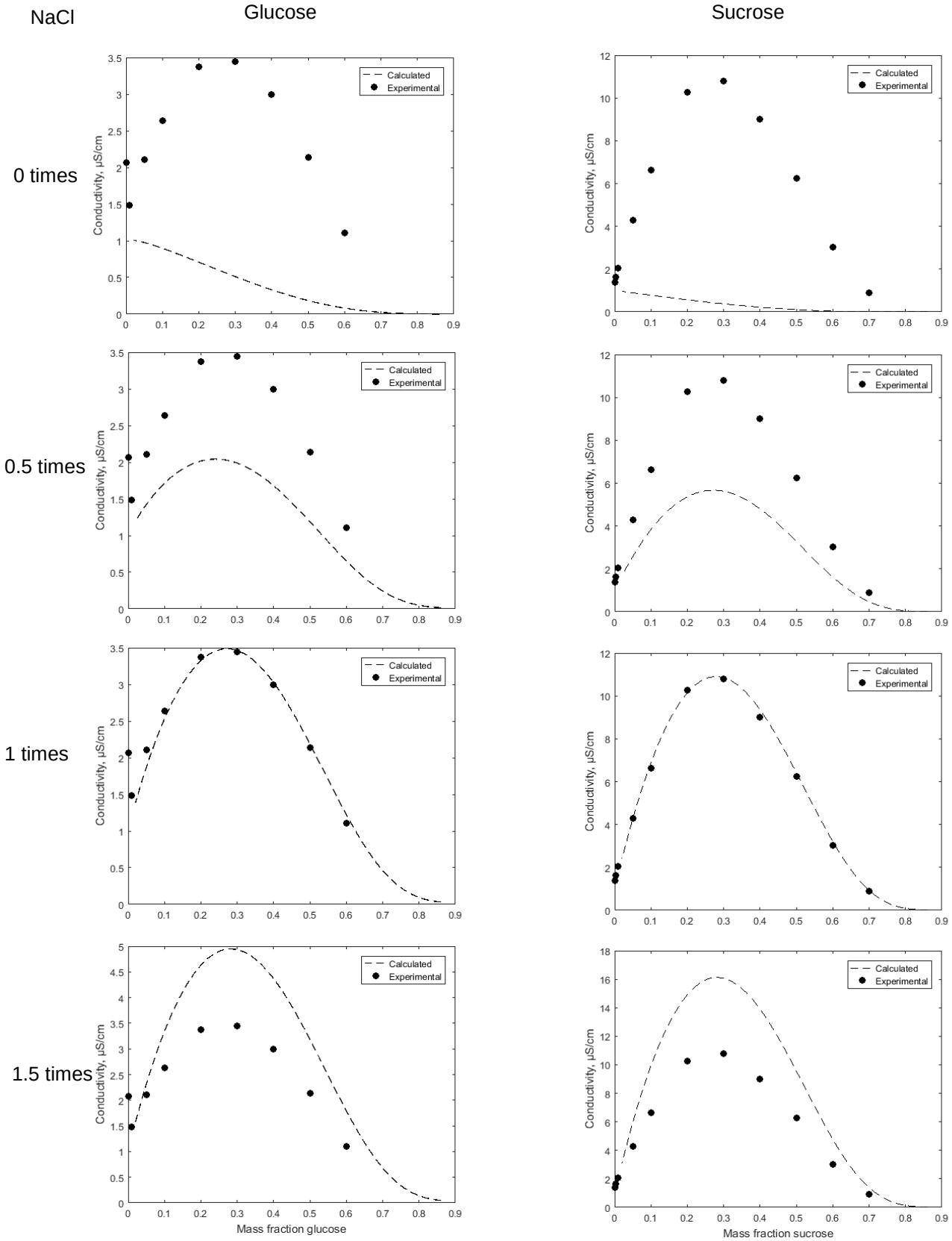


Figure 38 Sensitivities of the simulated values of electrical conductivities of binary sugar solutions expressed as zero to multiple factors of equivalent NaCl concentration. The experimental values are unchanged as obtained from a set of binary solutions. .

Figure 38 shows the variation in electrical conductivities at different salt concentrations for glucose and sucrose. The columns represent a particular sugar, each row represents the electrical conductivity of the sugar as a factor of salt concentration from 0 to 1.5 times equivalent NaCl. The concentrations of salts expressed as equivalent NaCl as a dry basis fraction of each sugar and were found to be 1.3×10^{-5} for glucose, 2.5×10^{-5} for fructose and 3.1×10^{-5} for sucrose. The calculated dashed line with 0 factor times minerals confirm that the dissociation of sugars is near zero. The other graphs show that conductivities are almost directly related to the relative concentrations of the salts. As evident from the experimental values in the graphs, salts present in the dry sugars that were used to prepare model honey solutions did influence the conductivity values but, no attempts were made to acquire very pure sugars. Simulation results showed that the salt content of the sugars would need to be at least 20 times smaller for there to be almost negligible effect of the salts on pure sugar conductivity.To obtain the fit shown in Figure 39, the value of α was changed to 0.735 which is close to the value of 0.78 found by Miller et al. (2000) for glucose up to 74% by mass. Using a value of 0.78 gave conductivity values about 30% lower than experimental values.

Figure 39 shows the conductivity of model honey solution with glucose, fructose and sucrose in typical honey composition of honey defined in Table 8. The variation in the predicted and measured conductivities of the model honey solution may be due to the experimental errors. The maximum value of absolute error observed was about 0.5 µS/cm at about 0.6 sugar mass fraction.

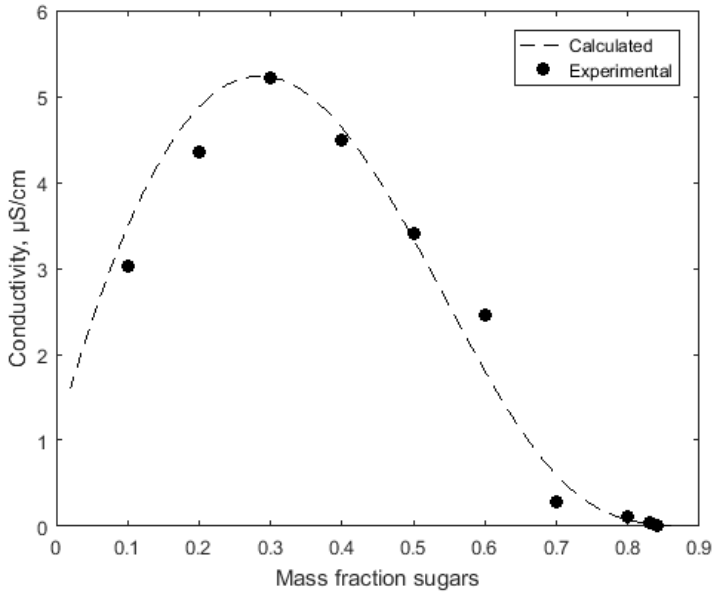


Figure 39 Experimental and calculated conductivities of model honey solution.

Figure 40 shows experimental and calculated conductivities using Equation 86-87 for model honey (17% water) with sodium chloride up to a mass fraction of 5% which goes beyond the maximum value of 1% ash reported by White et al. (1962). There is some indication of curvature in the data points which was also seen in preliminary trials using different solutions and a different conductivity sensor. A detailed analysis of the effects of sodium chloride on viscosity is required and hence numerous data points over a wider range is needed. However, the maximum relative deviation from the calculated value was about 0.067 µS/cm.

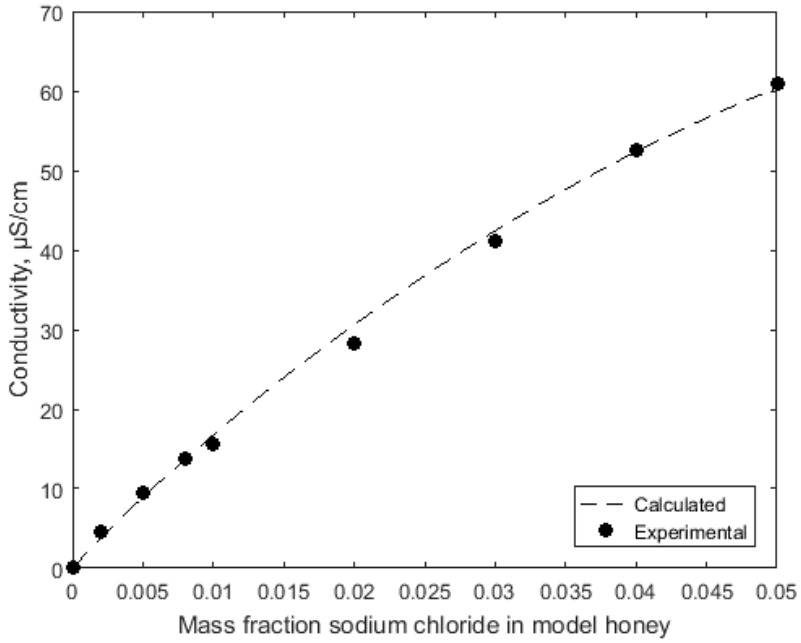


Figure 40 Experimental and calculated conductivities of model honey with 17% water with added sodium chloride using α = 0.735.

The conductivity of model honey with malic acid using a priori values and the revised α value of 0.735 was predicted to be higher than the experimental data. The calculations showed that H^+ was making the greatest contribution. A good fit (Figure 41) was obtained by increasing the pK_a for the first dissociation of malic acid from 3.46 to 3.58. The pKa for the second dissociation had no significant effect on conductivity. The maximum of 5% malic acid gives an acid concentration of about 750 meq/kg which is well above the maximum limit of 50 meq/kg set by Codex Alimentarius Committee on Sugars (2001).

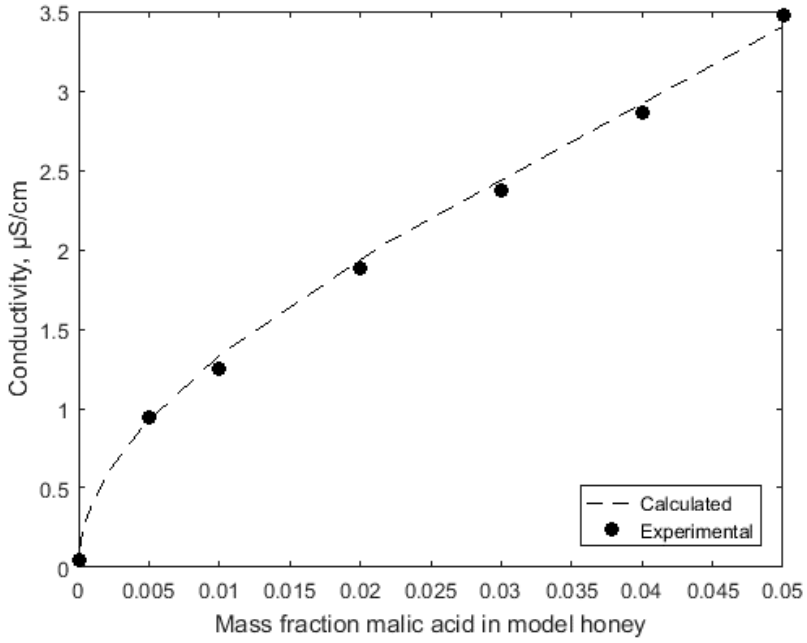


Figure 41 Experimental and calculated conductivities of model honey with 17% water and added malic acid.

Having made the parameter adjustments written above, the conductivity of model honey with 17% water, 0.5% NaCl and 0.5% malic acid was calculated and found to be 10.28 µS/cm. It was measured to be 10.34 µS/cm. This showed that there was no significant interaction effects between NaCl and malic acid. For this composition the individual ion contributions to conductivity are shown in Table 16. The contributions from dissociation of sugars, given by Equations (69 – 71), are not included because they are insignificant.

Table 16 The main contributions to conductivity in model honey.

	Conductivity contribution	
Ion	µS/cm	%
Cl^-	5.85	56.9%
Na^+	3.52	34.3%
H^+	0.868	8.4%
$malate^-$	0.036	0.3%

With a good set of parameters, it is possible to predict the effects of different variables on the conductivity of model honey. Figure 42 shows the predicted conductivity for model honey with 0.4% malic acid, 0.5% sodium chloride and water. The guidelines show the conductivity of the model honey system as per Codex 2001 standard for a 20% mass fraction of solids as 0.8 mS/cm. This shows the predicting capacity of the model proposed in this study.

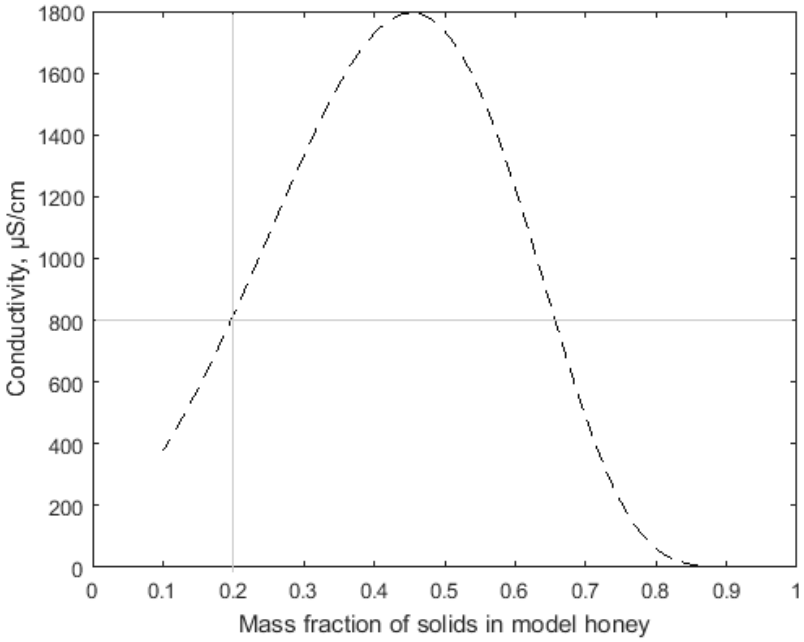


Figure 42 Predicted conductivity of a typical model honey system with glucose, fructose, sucrose, malic acid and sodium chloride at 20ºC

Figure 43 illustrates the effect of sodium chloride and water content on the predicted conductivity of model honey after is has been diluted for the standard test. This refers to Figure 43 over the normal range of water content, the effect of water on conductivity is much greater than the effect of doubling the salt content. This effect is almost entirely due to the strong influence of water content on viscosity when the water content is a typical value for honey. For example if the solids content of honey changes from 80% to 81% (water changing from 20% to 19%) the viscosity increases by about 56% giving a corresponding reduction in conductivity. It is for this reason that honeys are diluted to obtain a measure of the ash or mineral content.

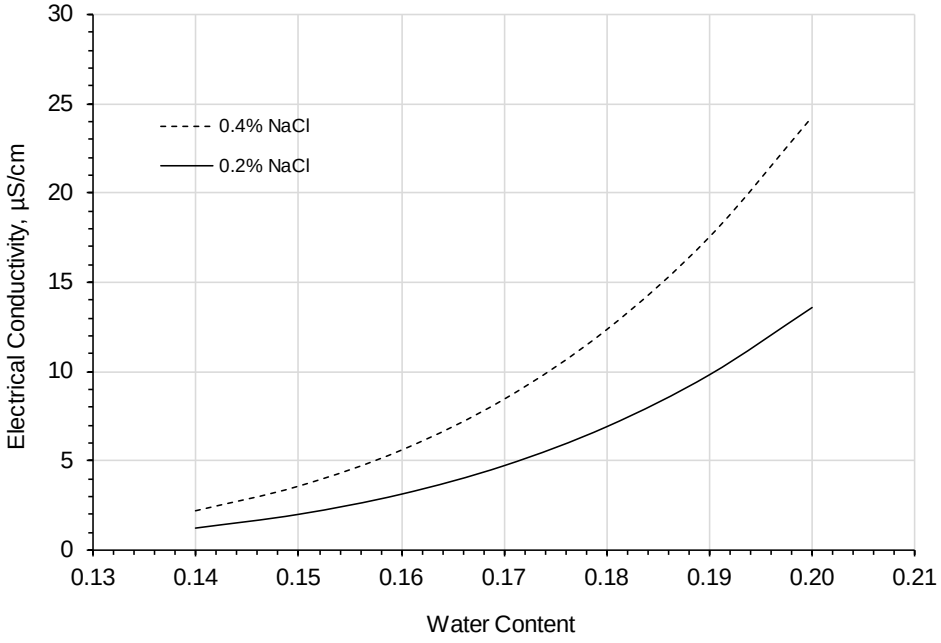


Figure 43 Predicted effect of NaCl and water content on the conductivity of model honey.

When honey is diluted by mixing 20 g of honey solids with 100 mL of water for the standard conductivity test, the effect of viscosity becomes very small so the conductivity is close to linear with salt concentration. If, for example, honey samples with 80% and 81% solids is diluted as required for the standard test, the difference in viscosity the difference between the two samples is predicted to be less than 0.5% and the resulting difference in conductivity is therefore small.

4.3.2. Viscosity and Electrical Conductivity of Model Food Liquids

(This section is based on the published article: Subbiah, B., Morison, K. R., 2018, Electrical Conductivity of Viscous Liquid Foods, Journal of Food Engineering, 237, 177-182.)

Many liquid foods such as milk, yogurt, and honey are complex solutions and colloidal dispersions containing sugars, proteins, minerals, fat and other minor components in water. Knowledge of property contributions by each component in milk will provide a clear understanding of the liquids characteristics (Madoumier et al., 2015). In this section, the relationship between electrical conductivity and viscosity is explored beyond sugar solutions. Sharifi & Young, 2012, 2013 established a correlation between conductivity and the composition of concentrated milk solutions with up to 47% solids. They used multiple linear

regression to relate the total solids content to temperature and electrical conductivity, but they did not consider the effect of viscosity.

St. Gelais and Champagne (1995) measured conductivity and viscosity of milk solutions as the pH was reduced. While their data show that both measurements changed, the changes appeared to be independent of each other. When the pH changed rapidly from 5.6 to 5.0, the viscosity increased about 100 times, while the conductivity only doubled. Henningsson et al. (2005) stated that proteins and lactose affected electrical conductivity of milk via viscosity, and that proteins, being charged, could contribute directly as a charge carrier.

A number of researchers have studied saccharide + electrolyte systems and proposed modifications to Walden's rule. Stokes (1959) confirmed that for KCl + sucrose solutions conductivity was inversely proportional to viscosity raised to the power of 0.7. Longinotti and Corti (2002) gave similar results for $MgCl_2$, $CaCl_2$, NaCl and KCl in trehalose and sucrose. More data is given for these chlorides in sucrose by Mitra et al. (2010).

Bordi and Cametti (1986) showed that the concentration (and hence the bulk viscosity) of carboxymethyl cellulose (NaCMC) has little effect on electrical conductivity when the concentration of $BaCl_2$ was greater than about 3 mmol/L. Below that concentration they use Manning ion condensation concepts to explain the effect of NaCMC concentration. This study was conducted to establish relationship for a range of food liquids at higher concentrations and hence higher viscosities. Model solutions were prepared using sugars, whey protein, milk powder, carboxymethyl cellulose and salts as in Section 3, and the electrical conductivity and viscosity of these was measured.

When conductivity is measured in a concentrated solution, the effective viscosity, which we refer to as "diffusion viscosity", η_{diff}, can be determined. It is known that the inverse relationship with viscosity in Equation (9) will fail when solutions are no longer dilute, but rather than use the modified Walden equation (Equation 10), it is proposed that an effective viscosity be determined. This will be the viscosity experienced by conducting ions as they diffuse under the influence of the electric field. In this work it is termed the "diffusion viscosity", η_{diff}, which can be estimated from the known ion concentrations and the measured conductivity. By rearranging Equation (87) we get

$$\eta_{diff} = \frac{f(\boldsymbol{C})}{\sigma_{conc}} \tag{88}$$

$$u_i = \frac{e_0 z_i}{6\pi\eta_{diff}^{\alpha} r_i} \tag{89}$$

Procedure to calculate diffusion viscosity

1. Obtain conductivities for commonly used salts, and the solution (water) viscosity at the same temperature. Hence get an expression for $f(C)$ in Equation (87) for each salt.
2. For mixed solutions, obtain conductivities at low concentrations with low viscosities and hence obtain estimates of the effective salt concentration on a dry basis.
3. At higher concentrations, use the dry basis ion concentrations to calculate the salt concentrations, then use the expected conductivity of the solution and the measured conductivity to calculate η_{diff}
4. Compare η_{diff} with η_{bulk}. Relate η_{diff} to the components that are likely to affect ion transport, i.e., estimate the composition of the solution through which ions move at a molecular scale.

The relationship between conductivity and viscosity with concentration for three different liquids is shown in Figure 44. Here there is a progression of behaviours. The conductivity of the NaCMC solution is linearly related to concentration and is hardly affected by the increasing viscosity. The conductivity increases due to the sodium counter-ions and also the residual NaCl normally present in NaCMC. However the long NaCMC molecules form a tangled network which increases the bulk viscosity, but within which ions can freely move. The molecular mass of the NaCMC was unknown but is typically about 10^5 g/mol.

The whey protein isolate (WPI) solution is similar to NaCMC but there is a reduction in the slope of the conductivity curve. The whey proteins in WPI are β-lactoglobulin and α-lactalbumin, with molecular masses of 18400 and 14200 g/mol respectively, and are small enough to interact more with ions thus reducing their mobility. Skim milk concentrate contains about 50% lactose and 40% protein on a dry basis so the resulting conductivity shows behaviour intermediate between the WPI solution and sucrose solution. The near-constant conductivity above 25% solids is most likely caused by the coincidental opposite effects on

increased ion concentration and increased viscosity. The conductivities of skim milk solutions were very similar to those obtained by Sharifi and Young (2012).

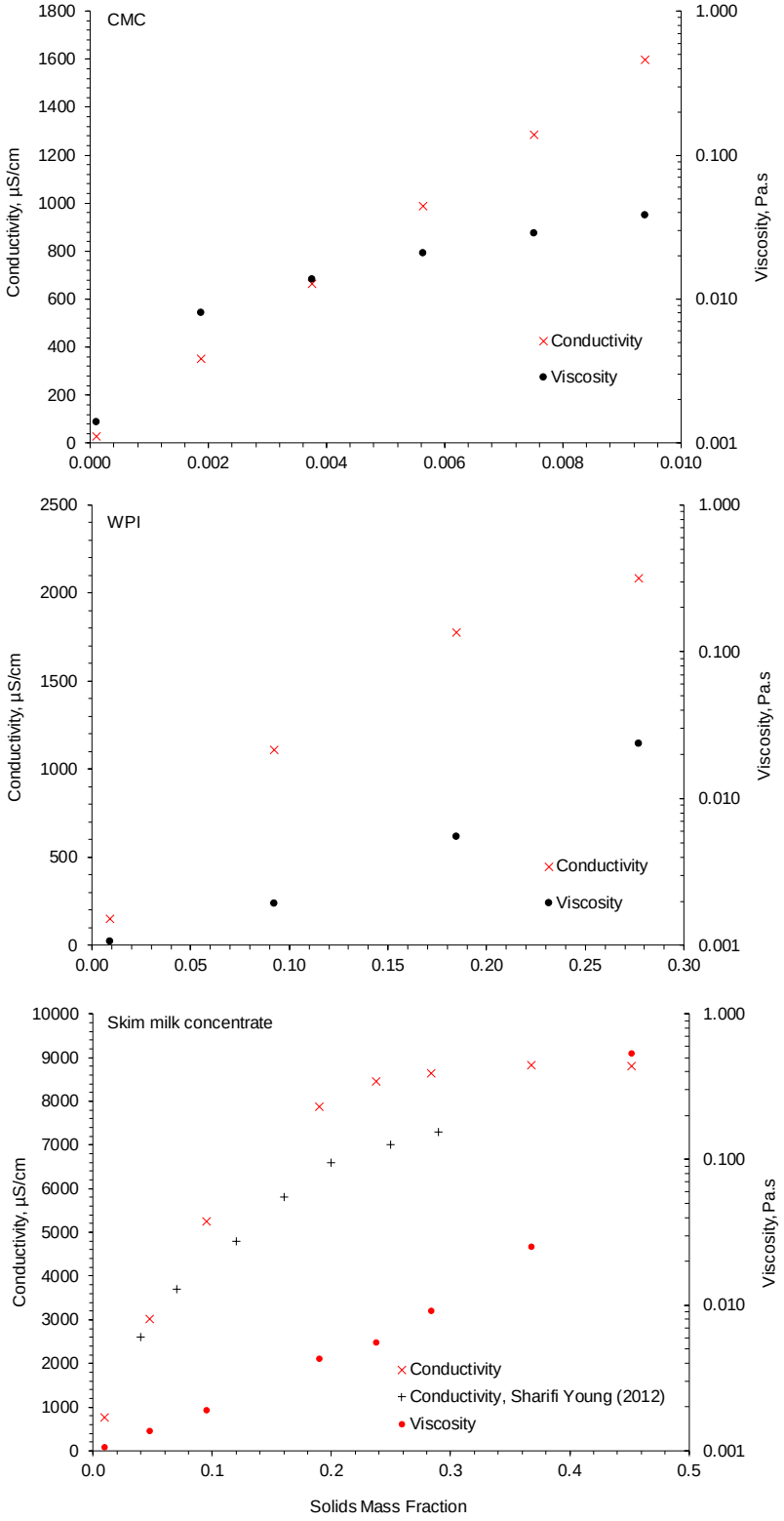


Figure 44 Electrical conductivity and viscosity of three different solutions: NaCMC, whey protein isolate & skim milk powder. Conductivities for skim milk solutions from Sharifi and Young (2012) are included. Note the logarithmic scale for viscosity.

If the modified Walden Equation (89) is applied to NaCMC, a best fit is obtained using $\alpha = 0.03$, which effectively shows the insignificant contribution of bulk viscosity. Instead the "diffusion viscosity" was calculated using Equation (88). First, using NaCMC as an example, the equivalent concentration of NaCl was fitted. Using an equivalent concentration of NaCl of 11.2% dry basis in NaCMC powder, the conductivity of the equivalent solution of NaCl, as plotted in Figure 45, was found to best match the conductivity of the NaCMC at low concentrations (where there was negligible viscosity effect).

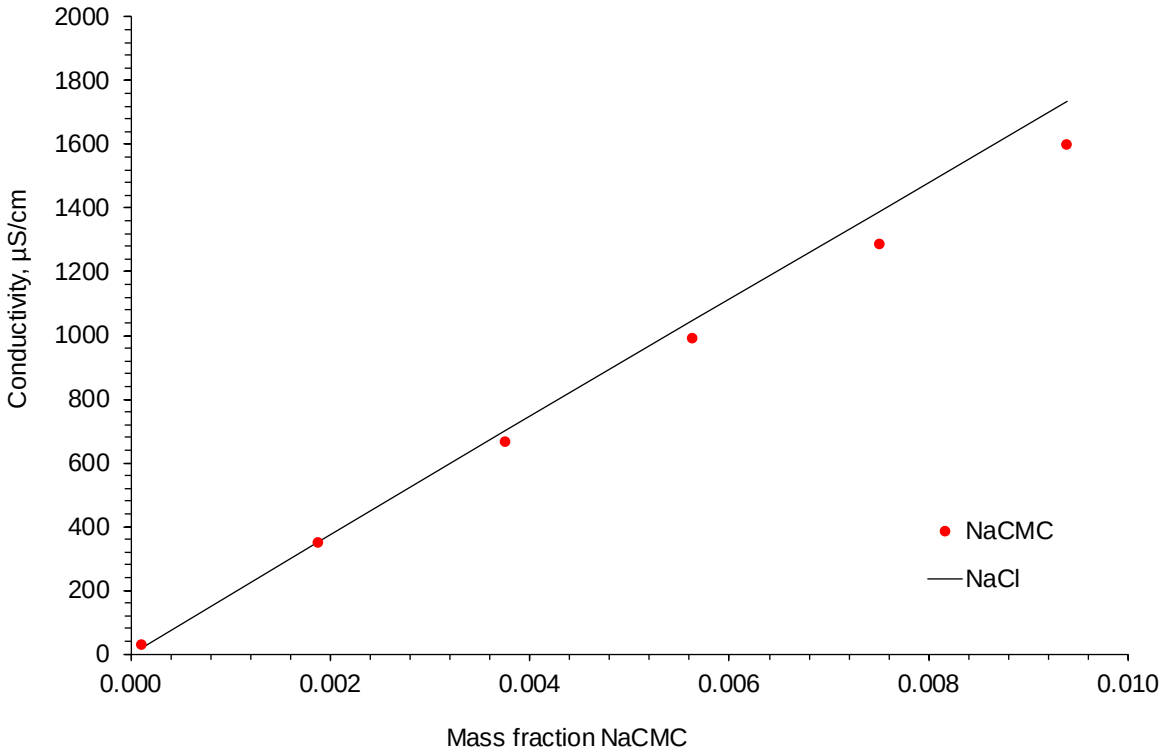


Figure 45 Conductivity of NaCMC solution and of an equivalent solution of NaCl fitted to be a tangent as concentration tends to zero.

Then using Equation (88) the diffusion viscosity was calculated and shown in Figure 46. The first point corresponded to a low conductivity with corresponding large error. The viscosity "experienced" by the conducting ions is shown to be very similar to that of water. Figure 46 shows two different sets of results obtained two months apart, indicating low experimental uncertainty.

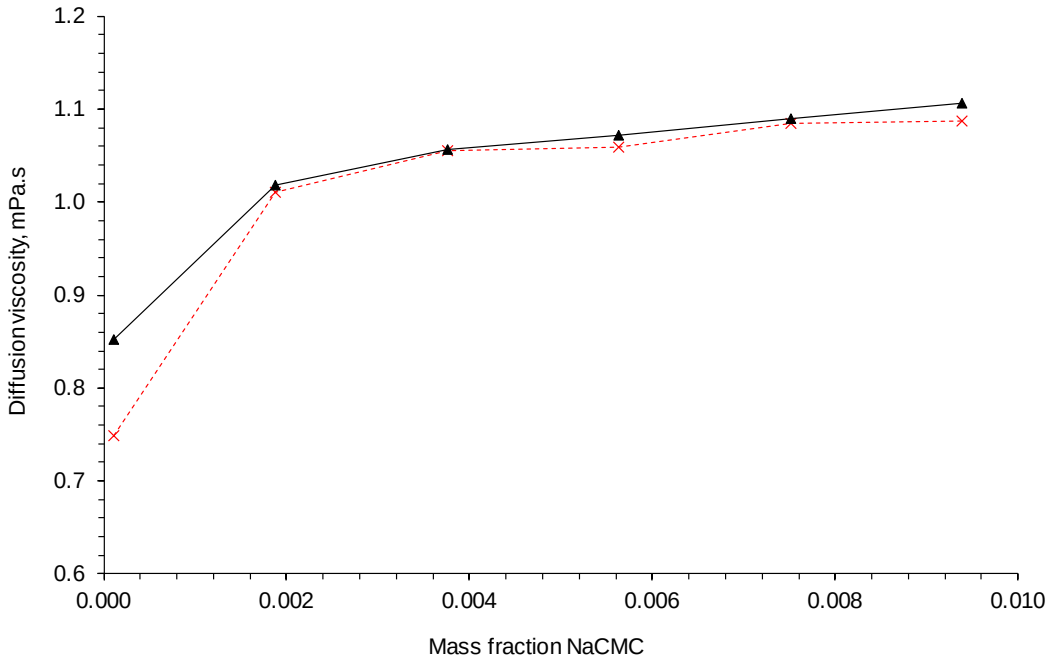


Figure 46 Calculated diffusion viscosity of NaCMC solutions from two different runs.

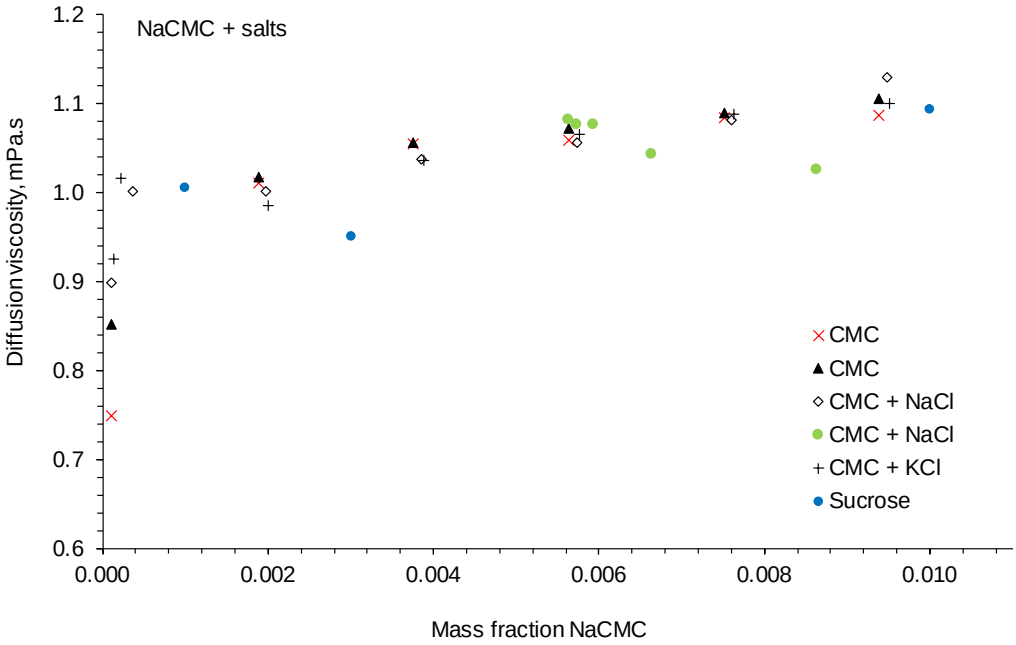


Figure 47 Calculated diffusion viscosity for NaCMC solutions. For comparison, results for sucrose are shown on all graphs. Note the log scale for sugars.

Figures 47 and 48 shows the result of similar calculations for other mixtures. The graphs show good consistency between similar solutions, but quite different results for different solutions.

The diffusion of ions through NaCMC solutions seems only slightly affected by mass fraction even though, as seen in Figure 44, the bulk viscosity increases significantly.

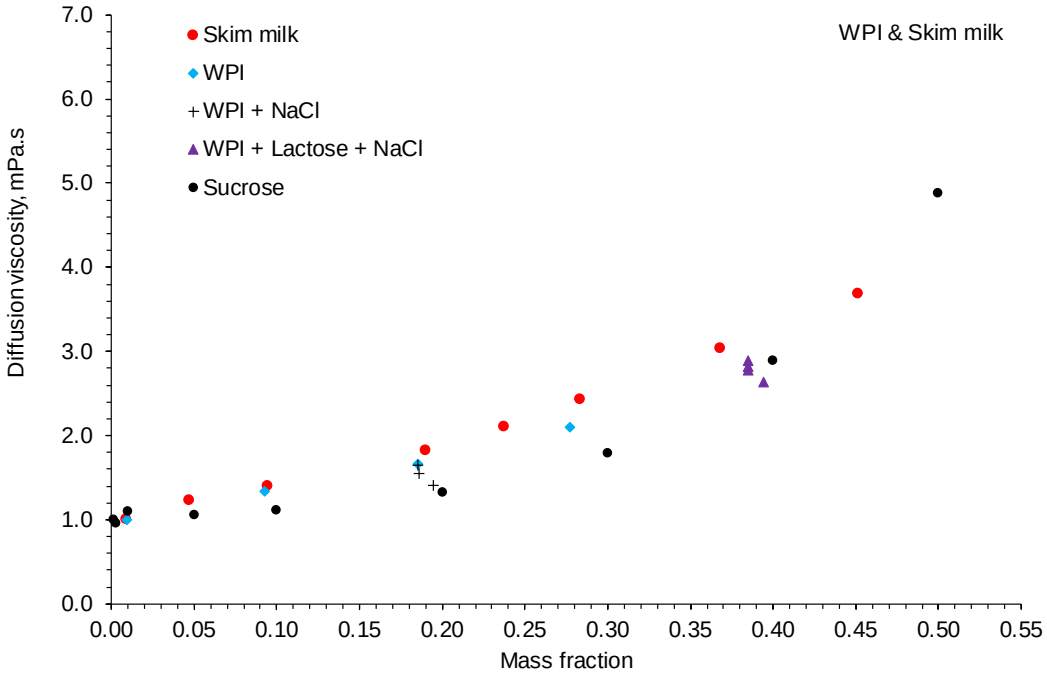


Figure 48 Calculated diffusion viscosity for WPI and skim milk solutions. For comparison, results for sucrose are shown.

Figures 47 and 48 shows the result of similar calculations for other mixtures. The graphs show good consistency between similar solutions, but quite different results for different solutions. The diffusion of ions through NaCMC solutions seems only slightly affected by mass fraction even though, as seen in Figure 44, the bulk viscosity increases significantly. Here the mass fractions are very low, but concentrations higher than about 1.5% are difficult to achieve. The diffusion viscosities for WPI and skim milk are higher than for sucrose at similar concentrations, showing greater resistance to ion flow. This is contrary to expectations as WPI and milk solutions have a lower bulk viscosity than sucrose at the same concentration. It is likely that there is significant interaction between ions and the protein which will have a negative charge in water.

The ratio of the diffusion viscosity to the bulk viscosity was calculated and is shown in Figure 49. The relationship for sugars, WPI and skim milk is surprisingly linear with concentration, but there is no obvious reason for this. The significant difference between diffusion viscosities is not suitable for use in Walden's equation at high viscosity.

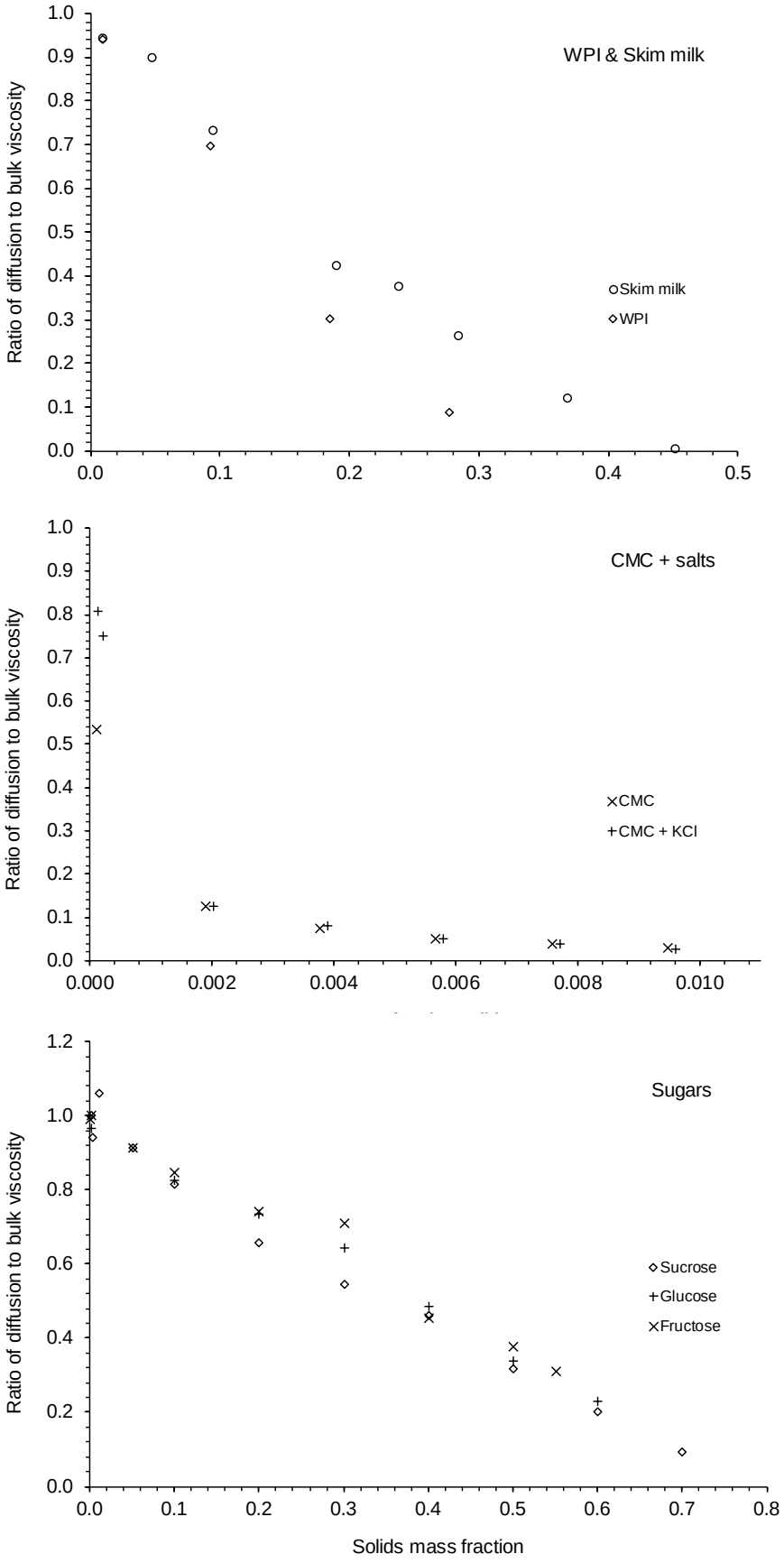


Figure 49 The ratio of calculated diffusion viscosity to bulk viscosity for various solutions.

The results presented so far do not lead to a prediction equation for electrical conductivity. While the modified Walden's rule can be applied for pure sugar solutions, the effect of bulk viscosity and conductivity can probably be ignored in solutions of long chain polymers such as NaCMC. In other solutions this effect cannot be predicted.

4.4. Refractive Index

The rearranged Redlich-Kister equation (Equation 34) as rewritten below will be used to fit the refractive index data.

$$n^2 = (\phi_A (n_A^*)^2 + \phi_B (n_B^*)^2) + \phi_A \phi_B (A_0 + A_1 (2\phi_A - 1)) \quad (34)$$

When Equations (32 - 34) as given in the literature review are combined there are three fitting parameters: n_B^*, A_0, and A_1.

Data is given by Jackson and Mathews (1928) for fructose solutions up to 89% by mass, with resolution of 5 decimal places. They were fitted by non-linear least squares giving $n_{fructose}^* = 1.53524$, $A_0 = 0.06944$, and $A_1 = -0.03403$. The difference between the fitted and experimental values which is well within ± 0.0002 are as given in Figure 50. The random nature of the differences is an indication of a better fit and indicates that adding another term to Equation (32) will not be necessary. It is noticeable that the differences are larger at higher solution concentrations. This was found to be the case for all sugars, but it is not clear if there is a problem with experimental accuracy, or possibly a change in dissolved solute structure at concentrations close to saturation.

Data from Fucaloro et al. (2007) for fructose up to 38% gave deviations of refractive index up to 0.0003 compared with the maximum difference of 0.00008 for the Jackson and Mathew data in the same concentration range. The following parameters were obtained for other sugars using data from Weast (1978).

Table 17 Parameters for prediction of refractive index using Equation (34)

Sugar	Apparent density infinite dilution	n^*	A_0	A_1
Glucose	1617.82	1.53733	0.04842	-0.01926
Fructose	1635.24	1.53524	0.06944	-0.03403
Sucrose	1624.86	1.54555	0.03639	-0.02848
NaCl	1180.64*	1.52506	0	0
Malic acid	1640	1.4747	0.1116	0.00551

* For sodium chloride the apparent density was allowed to vary to fit the data. A_0 and A_1 were set to zero with no loss of fit.

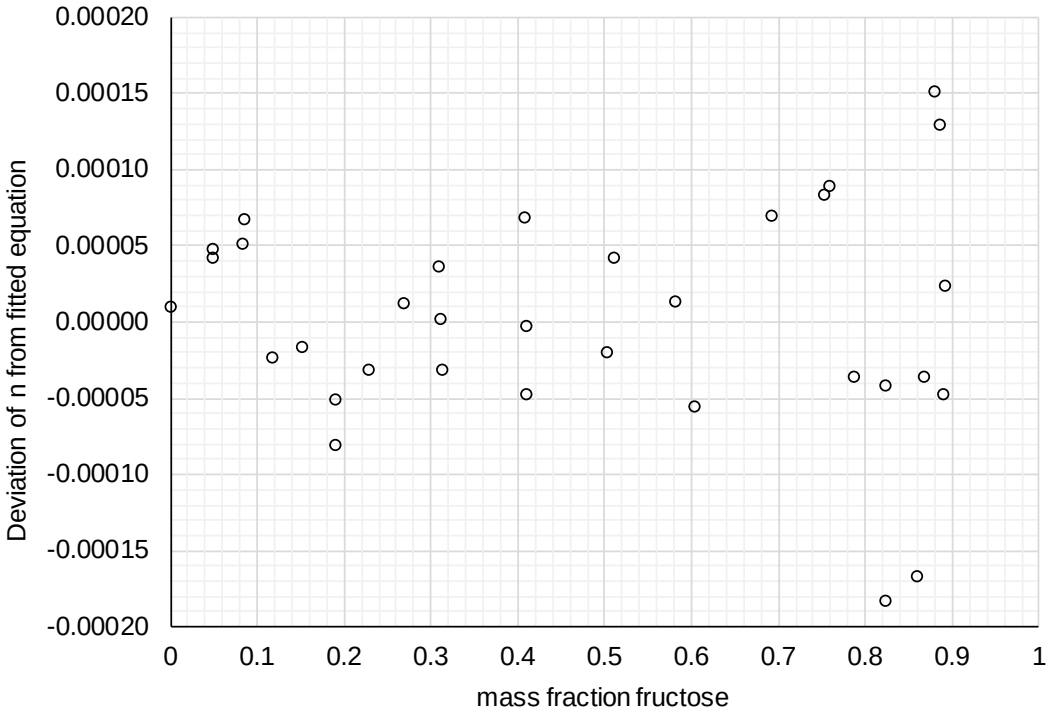


Figure 50 Deviation of the measured refractive index to the calculated refractive index

It is assumed that the ideal mixing rule (Equation 29) can be applied and that there is no further excess refractive index due to the interaction of different components. This is unlikely to be true, but is a useful assumption to avoid the need to test all possible mixtures. Thus instead of mixing pure A and pure B, a ternary solution can be considered as a mixing a solution of A and a solution of B. This yields:

$$n_{mix} = \sqrt{\frac{\frac{w_A}{\rho_A}(n_A)^2 + \frac{w_B}{\rho_B}(n_B)^2}{\frac{w_A}{\rho_A} + \frac{w_B}{\rho_B}}} \tag{90}$$

Here w_A and w_B are the mass fractions of A and B in the ternary solution, and the densities and the refractive indices are the properties of binary solutions determined at the total mass fraction of the solution, $w_A + w_B$. Equation can be generalised for multiple components as

$$n_{mix} = \sqrt{\frac{\sum_i \frac{w_i}{\rho_i} (n_i)^2}{\sum_i \frac{w_i}{\rho_i}}} \tag{91}$$

Here i represents a binary solution of component i and a common solvent. Hence the solvent is not explicitly included in Equation (91).

Data for the refractive indices of invert sugar, an equimolar solution of glucose and fructose in water, was given by Snyder and Hattenberg (1963). The differences between the refractive indices of the mixture and the individual components is as shown in Figure 51. These difference show a consistent pattern like cubic spines. The refractive index of invert sugar didn't deviate in any consistent pattern. However, the difference calculated from refractive index data of invert sugar and that of the individual sugars shows a consistent pattern and this needs to be further studied.

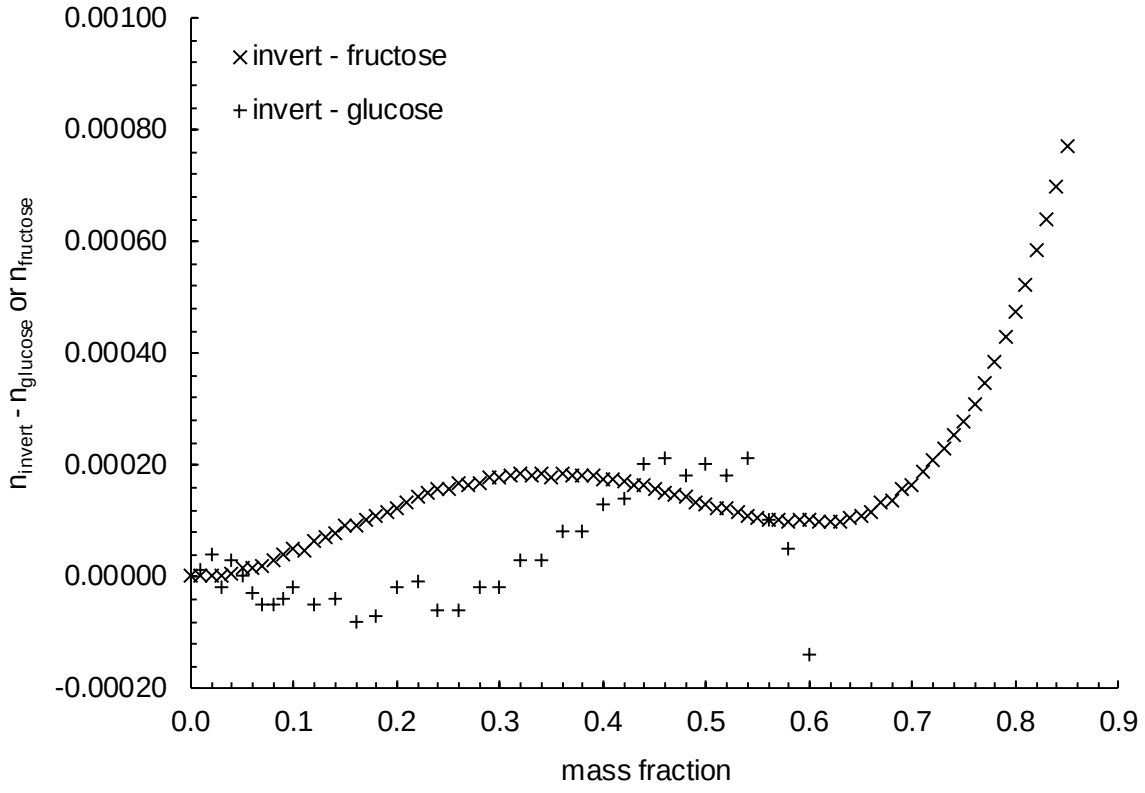


Figure 51 Difference between the refractive indices of invert sugar and fructose and glucose at 20 °C.

Equation (91) was applied to mixtures of glucose + fructose + water with added sodium chloride and/or malic acid. Figure 52 shows discrepancies of up to 0.0014 between measured and predicted values. The measured refractive index with added sodium chloride changed at a greater rate than expected from Equation (91) indicating a greater interaction between the solutes than expected from ideal mixing. In contrast malic acid had a greater negative effect than expected.

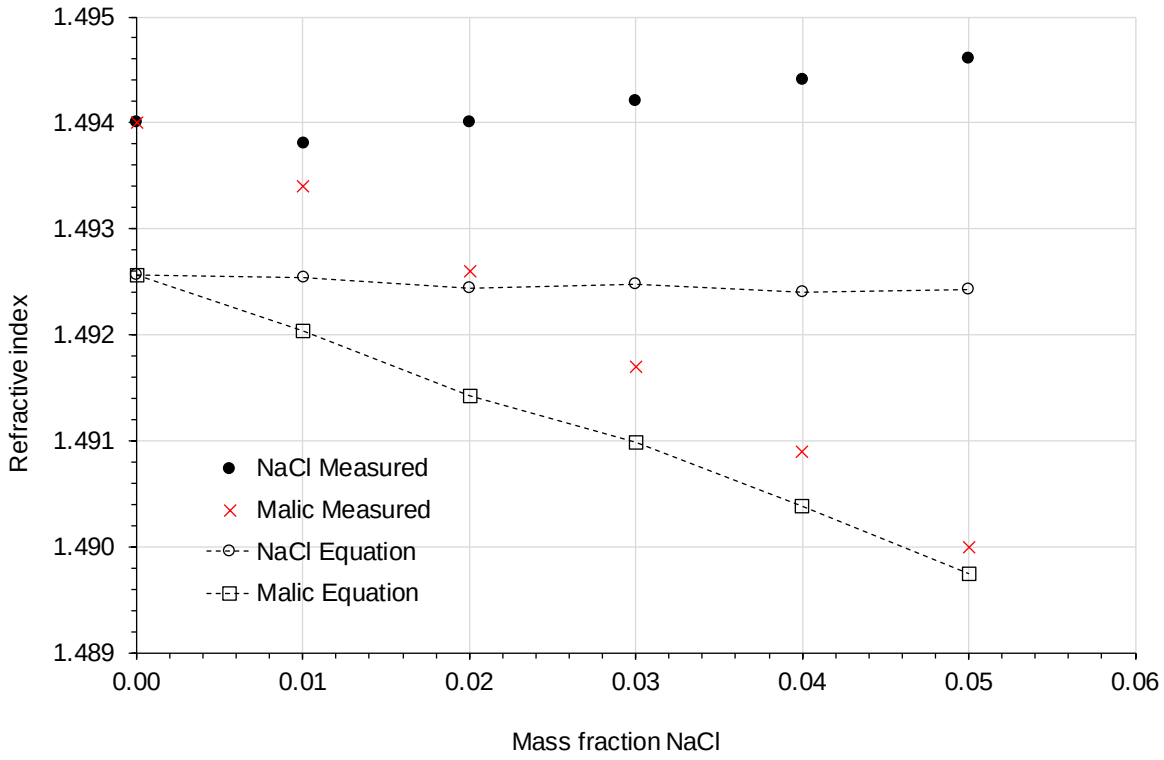


Figure 52 Experimental and prediction refractive index values of glucose + fructose solutions with added NaCl or malic acid with 83% solids at 20 °C (From compilation, model honey systems).

Table 1 for the composition of honey indicates a maximum of 1% salts (ash) and 1.2% acids. Hence it is likely that the maximum effect on refractive index is almost zero from salts and perhaps -0.0005 from the acids, if they behave the same as malic acid. This is not inconsistent with the Codex RI values for honey which are about 0.001 above the RI of the same mass fraction of invert sugar.

4.5. Water Activity

Water activity data for sucrose was extensively reviewed by Starzak, Peacock and Mathlouthi (2000) and is effectively summarised by Equation (54). The hydration numbers calculated from this are shown for different temperatures in Figure 53. Here hydration number is related to the mass fraction of water, as this can be more easily extended to systems in which mole fractions cannot be easily determined. Further the fraction of water, rather than the fraction of sugar is used, because as will be discussed later, in mixed systems the hydration is related to the amount of water, not the concentration of any individual solute. This graph provides a useful indication of the effect of temperature that will be used in later discussion.

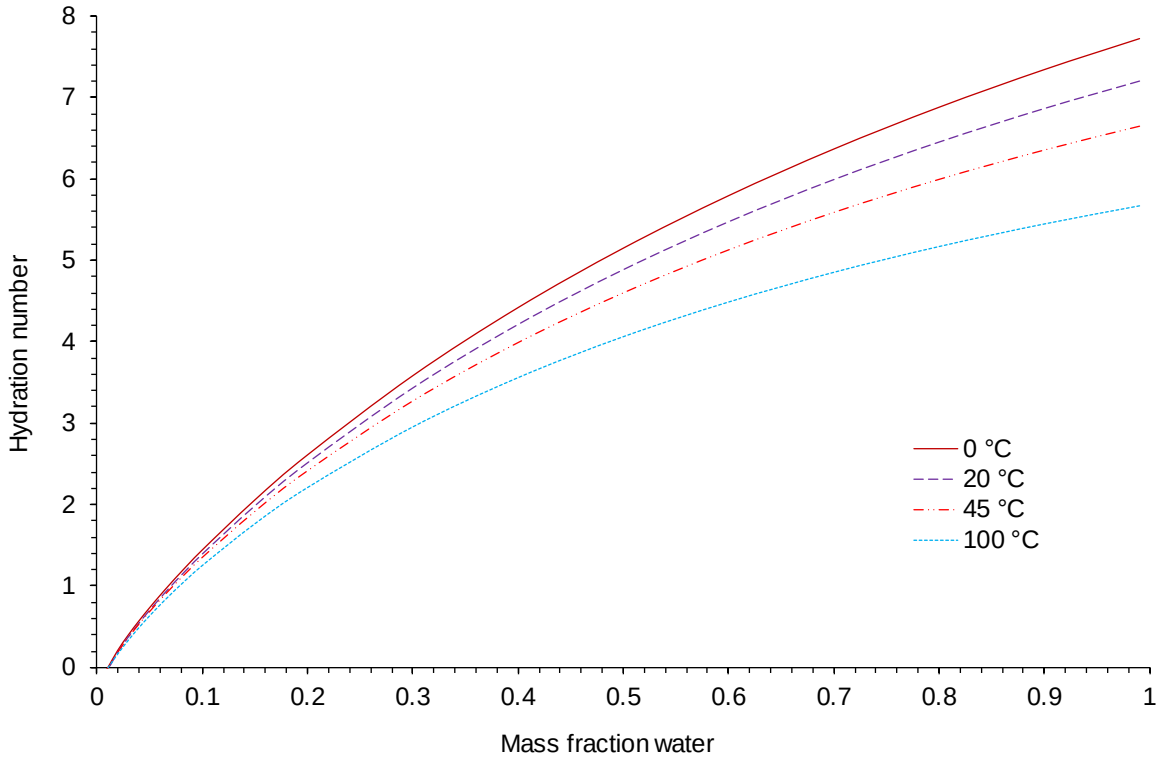


Figure 53 Hydration numbers for sucrose using Equation (51) proposed by Starzak, Peacock and Mathlouthi (2000) over a range of concentration as a function of temperature

Useful data for glucose water activities is given by: Bhandari and Bareyre (2003) who used a water activity meter with a sensitivity of 0.001; Miyajima et al. (1983), isopiestic measurements; Bonner and Breazeale (1965), isopiestic measurements; Stokes and Robinson (1966), isopiestic measurements; Taylor and Rowlinson (1955), vapour pressure; Cooke et al. (2002a), vapour pressure; and Zamora et al. (2006b), water activity meter. Ross (1975) used data from the Corn Industries Research Foundation (1969). All these data were converted to

hydration number and are shown in Figure 54. It seems very unlikely that the hydration number should reduce in more dilute systems, and as pointed out by Starzak et al. (2000) the value of hydration number is extremely sensitive to experimental error in dilute solutions. Therefore the low values of Bonner and Breazeale (1983), and of Miyajima et al. (1965) in dilute solutions were ignored. The increase in hydration number with temperature given by Cooke et al. (2002a) is contrary to the expected decrease according to Equations (54) and (53) as shown by Figure 53 for sucrose. This discrepancy casts doubt on the accuracy of this data. Hydration number for the data of Velezmoro et al. (2000) was calculated and ignored as only two data points were close to feasible.

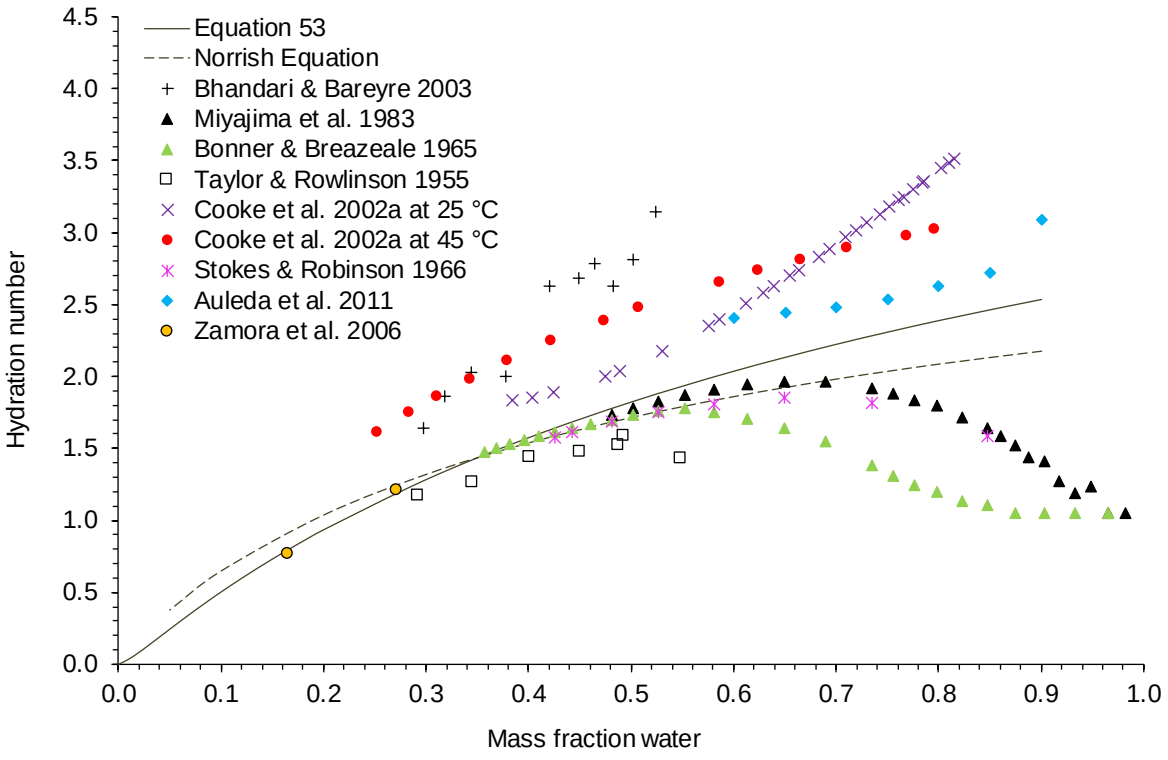


Figure 54 Hydration number of glucose compared to literature data. Water activity calculated using Equation (54) with Q = –6500 J mol-1, b_1 = –1.0 and b_2 = 0.1 and hydration number calculated using Equation (53)

In Figure 54 hydration numbers calculated from the data for glucose are plotted *vs*. water mass fraction. The parameters for Equation (54) for glucose were fitted by eye based guided by the parameters for sucrose. It is acknowledged that a low water content is not physically feasible without supersaturation. The model curve is expected to pass through the origin as a positive y-axis intercept is impossible as this requires hydration without water, and a positive x-axis intercept seems unlikely as very small fractions of water are likely to be strongly attracted to

the sugars. The data showing a reduced hydration number at high water fractions contradict the statement by Stokes and Robinson (1966) that the average hydration number per solute molecule will reduce with increasing solute concentration.

Consider a 0.0001 change in water activity measurement for sucrose solutions, a 2.5% error in the hydration number calculation is shown and hence shows the sensitivity of the water activity measurements for sucrose as shown in Figure 55. From the above error calculation, it is understood that for $w_{sucrose} \geq 0.4$, the $\frac{d\bar{n}}{da_w}$ can be understood. The $d\bar{n}$ from a 0.0001 change produces an error of 0.06 and $d\bar{n}$ for 0.0005 for a change produces an error of 0.3 and hence the error in $\bar{n}$ is becoming less significant.

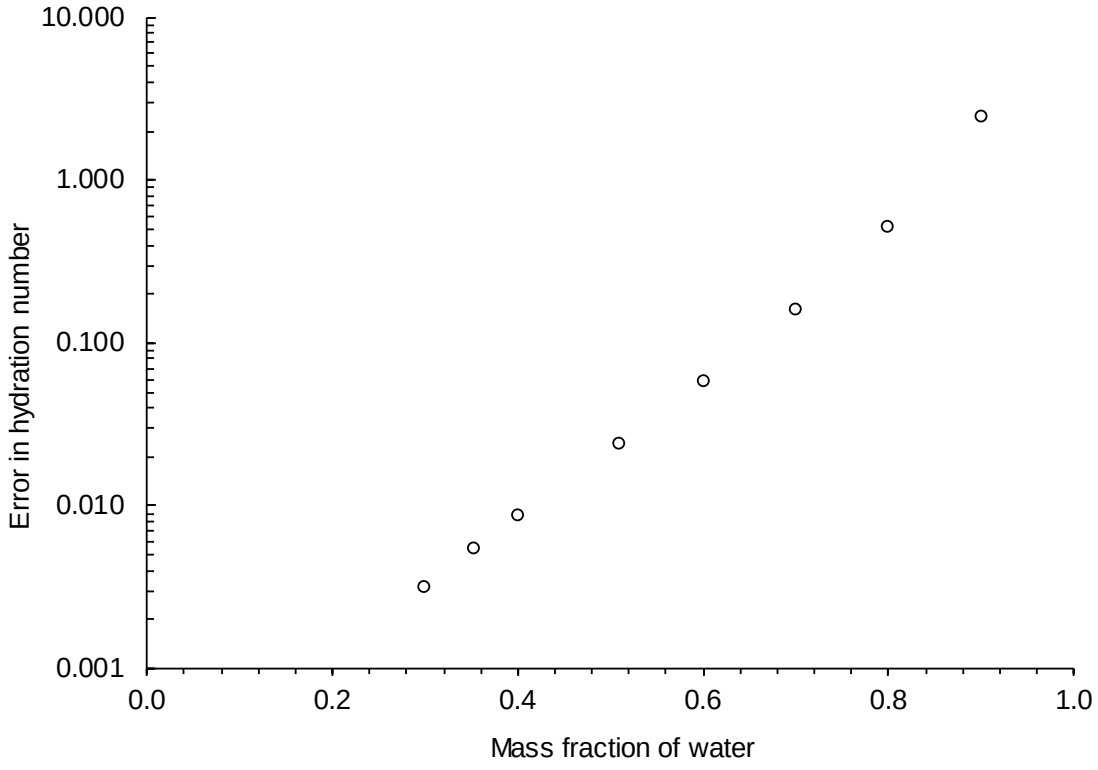


Figure 55 Sucrose water activity sensitivity. Error calculated for the hydration number using a 0.0001 change in water activity

The available data for fructose are given in Figure 56. Cooke et al. (2002) gives data for the vapour pressure of fructose solutions at 45 °C. It is apparent that the last two data points for fructose given by Cooke et al.(2002) have been typed incorrectly. However given the deviation of Cooke's data points from other data, it seems unwise to rely on it. However, if Cooke's data for fructose and glucose have consistent errors, it does indicate that the hydration number of

fructose is lower than that of glucose. Gharsallaoui et al. (2008) found hydration numbers for fructose from data of Cooke et al (2002), but their values (2008) do not correspond with the values calculated directly indicating a calculation error.

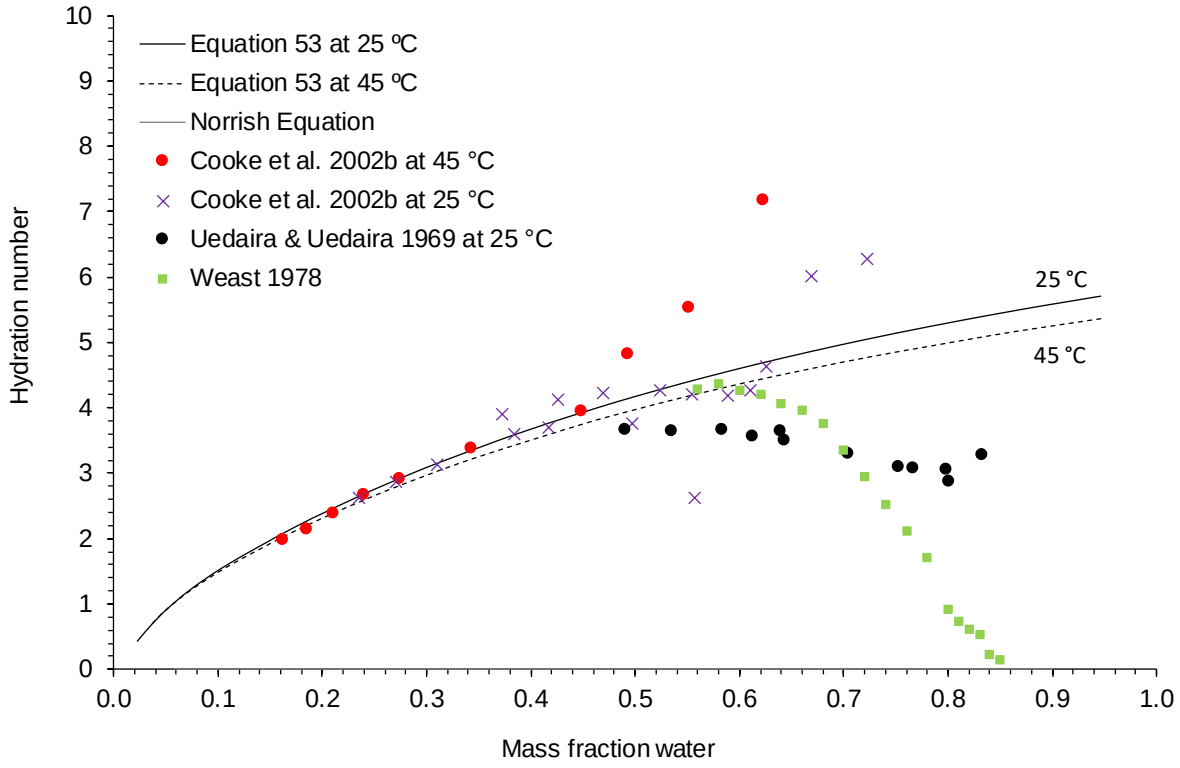


Figure 56 Hydration number of fructose compared to literature data. Water activity calculated using Equation (54) with Q = – 5500 J mol-1, b_1 = – 0.5 and b_2 = – 0.25 and hydration number calculated using Equation (53)

Zamora et al. (2006a) obtained very similar water activities for glucose and fructose, with the hydration number of glucose being about 0.05 higher than fructose. Auleda et al. (2011) gave freezing point depression for glucose, fructose and sucrose. The calculated hydration number for fructose was about 0.4 lower than for glucose at the same concentration. Velezmoro et al. (2000) also showed lower values for fructose but data for less than 30% solids is very unreliable.

Maltose data were given by Cooke et al. (2002a) from vapour pressure, Uedaira and Uedaira (1969) from isopiestic measurements and Weast (1978) are shown in Figure 56. Clearly more data is desirable. . Data (of unknown origin) given by Weast (1978) show a discontinuity at about 21% by mass of maltose and clearly show the large uncertainties at lower sugar concentrations.

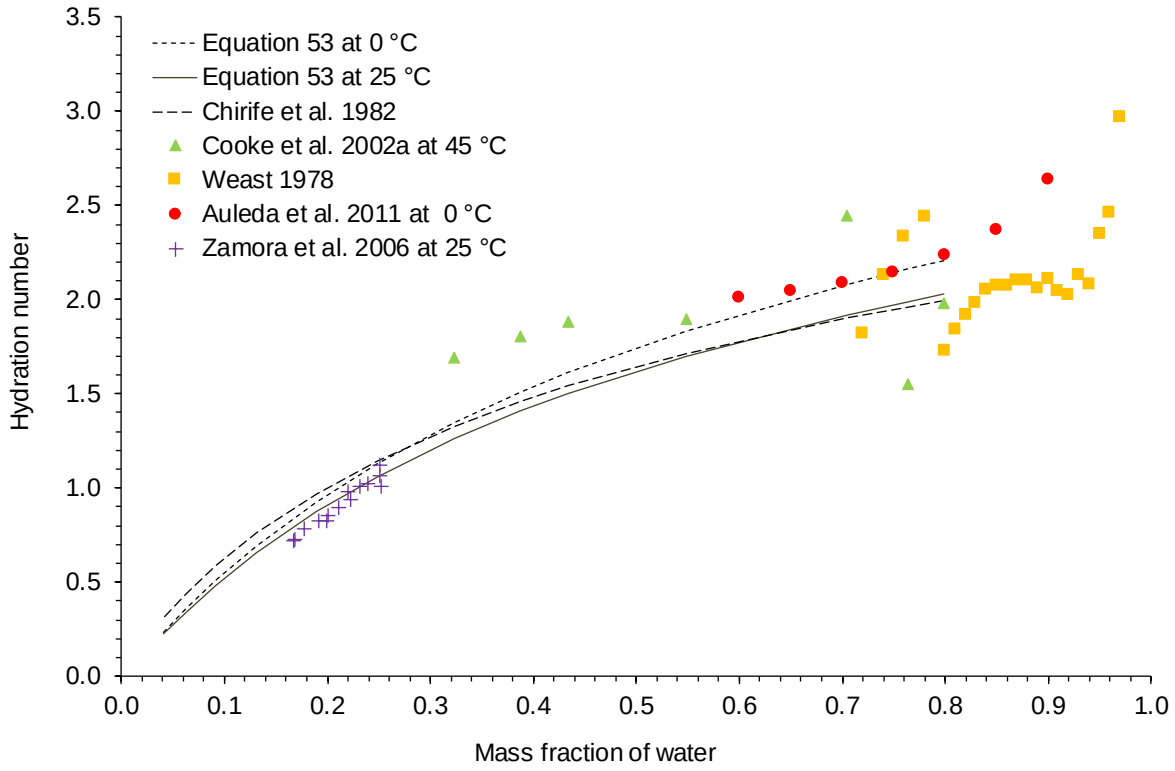


Figure 57 Hydration number of Maltose compared to literature data. Water activity calculated using Equation (54) with Q = – 14500 J mol-1, b_1 = – 1.0 and b_2 = 3.0 and hydration number calculated using Equation (53).

Gharsalloui et al. (2008) claimed that for trehalose, the hydration number should intersect with the composition of trehalose dihydrate. They did not present compelling evidence that the liquid and solid phases should intersect in this way.

The same approach worked well for glycerol using vapour pressure data from To et al. (1999)as can be seen in Figure 58. The data of Chenlo et al. (2004) did not yield consistent or realistic values of hydration number and is not included. Marcolli and Peter (2005) showed consistent deviation in the lower water mass fractions and high deviations above 45% mass fraction from the fitted values using Equation (54). The data from Ninni et al. (2000) showed good fit up to 55%mass fraction and deviated over this water fraction. The data form Scatchard et al. (1938) was a fair fit up to 65% water fraction, but at higher water fraction there appeared a consistent deviation from the fit and this may be attributed to the measurement technique employed. Weast (1978) data was totally inconsistent with the literature data set available and not a feasible to use. Hence the data was discarded form any analysis.

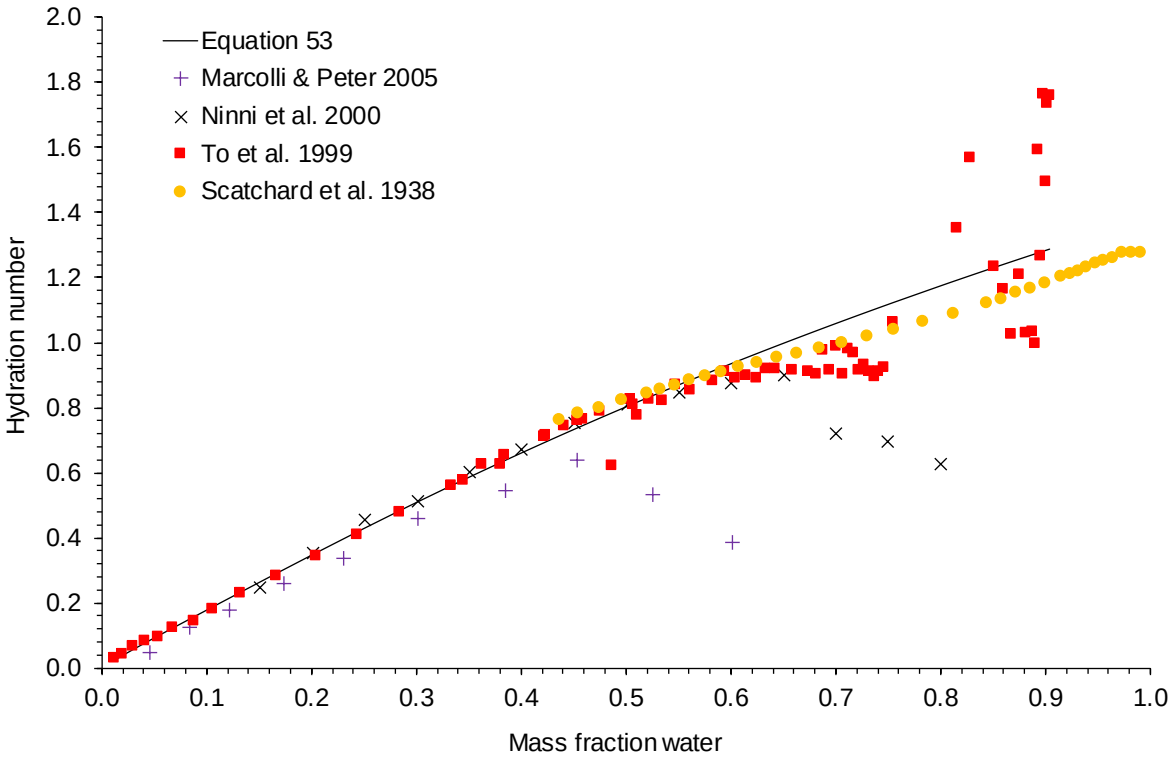


Figure 58 Hydration number of Glycerol compared to literature data. Water activity calculated using Equation (54) with Q = – 3435 J mol-1, b_1 = – 1.27 and b_2 = 0.65 and hydration number calculated using Equation (53).

The data for sorbitol are somewhat unsatisfactory. Analysing the available data, the data are scattered and contradict the statement of Stokes and Robinson (1966). The experimental errors cause an unlikely reduction in calculated hydration number at low sorbitol concentration. Additional data points are required to analyse the validity of the fitted equation. Even if a better equation is found, this analysis shows that the data are very inconsistent.

4.5.1. Model Food Systems with Sugars, Salts and Acids

Sereno et al. (2001) detailed the different models that are available to calculate the water activity of aqueous solutions relevant to food systems. However, they fail to acknowledge the extensive review by Starzak et al. 2000 which adopted the hydration theory in sugar solutions relevant to food systems as approached by Scatchard. The current research acknowledges this approach and hence extends the approach to food systems with multiple, mixed solutes.

One approach is the Ross (1975) equation (Equation 38) which gives the water activity of a mixture as the product of the component water activities calculated using the component water activities evaluated in binary solutions at the same molality as in the mixture. The Ross model will be used as a comparison to the proposed model. The shortcomings of the other two models as discussed in the literature review in Section 2.3.5 for multi-component systems are applicable and hence will not be used for comparison.

It is proposed here that Scatchard's approach as discussed in Section 2.3.5 and represented as Equation (50), and Equation (51) can be extended to mixtures of sugars by using the same idea used by Scatchard that the water activity is the mole fraction of active water. Wang et al. (2016) concluded that the free water in the system effectively is measured by water activity. In this equation the hydration numbers are calculated for binary solutions at the same mole fraction of water as the mixture. The use of mole fraction rather than mass fraction allows extension to systems with electrolytes that dissociate.

$$a_w = \frac{c_w^o - \sum \bar{n}_i c_{s,i}^o}{\left(c_t^o - \sum \bar{n}_i c_{s,i}^o\right)} \tag{92}$$

$$a_w = \frac{x_w^o - \sum \bar{n}_i x_{s,i}^o}{\left(1 - \sum \bar{n}_i x_{s,i}^o\right)} \tag{93}$$

Here, c_w° is the nominal water concentration, $c_t^\circ$is the total concentration of the solution, $c_{s,i}^\circ$ is the concentration of the i component (sugar or salt), $\bar{n}_i$is the hydration number of the i component, x_w° is the mole fraction of water and $x_{s,i}^\circ$ is the mole fraction of the solute component i in the food mixture.

The approach from binary systems has been extended to multi-component sugar food systems, the model was applied to a binary sucrose solution, assigning values of zero to all other components.

Rüegg and Blanc (1981) obtained water activity measurements of model honey solutions using an electronic hygrometer. They used water fractions from 12.1% to 28% with a dry-basis composition of 48% fructose, 40% glucose, 10% maltose and 2% sucrose. Their results together with the predictions from Equation (93) are given in Figure 59. Calculations were also made with Ross's equation using binary water activities calculated from Equations (51 – 53)

in this work. The agreement with Equation (93) at moistures above 20% is good, but the bottom two data points show positive deviations from any smooth curve. Possibly the sugars were not fully dissolved, or there are deviations due to the high degree of supersaturation. Ross's equation was much less effective at this moisture content. Gaida et al. (2006) also attempted to fit this data, but the deviations are much higher than those shown here.

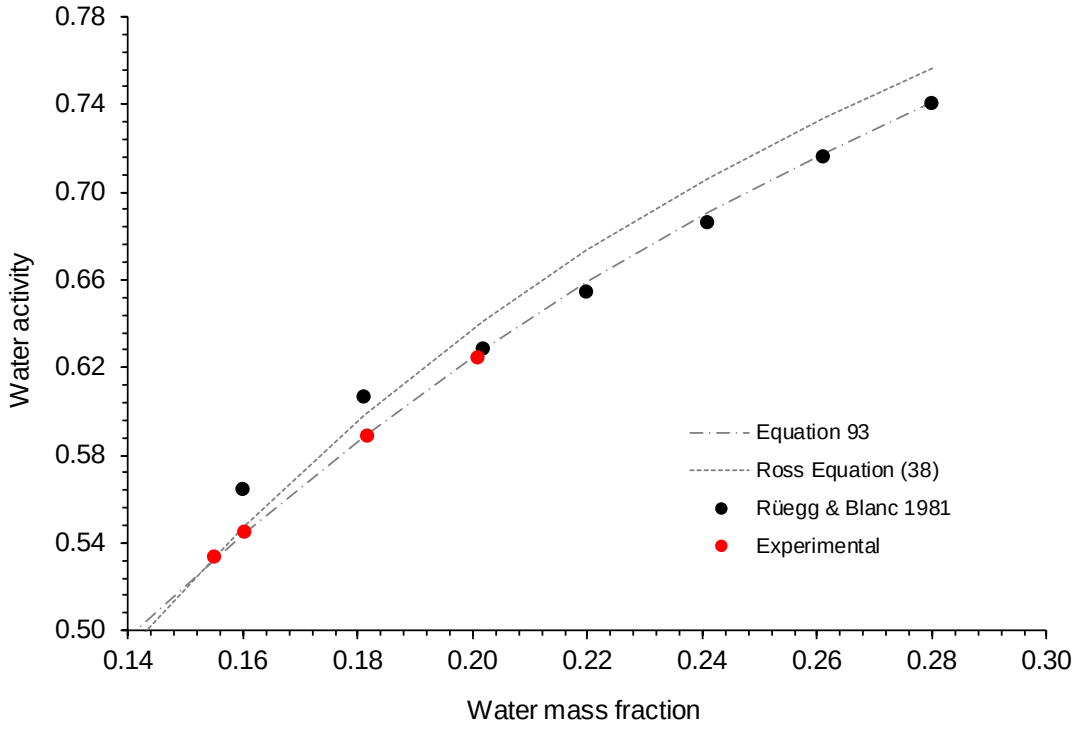


Figure 59 Water activities of model honey from Rüegg and Blanc (1981) with Equation (93) and Ross's equation

Considering another model food system analysed by Fontan et al. (1981), the experimental data is compared to the proposed Equation (93). Apple juice was studied as a model food system comprising of different sugars at dry basis mass fractions of 62.52% fructose, 14.75% glucose, 22.73% sucrose. Figure 60 shows the deviation in experimental values from the fitted equation. A consistent error in the measurement is visible and a maximum deviation is observed at 60% sugar mass fraction of 0.006. Contrary to the claims of meter calibration in the study reported by Fontan et al. (1981), the consistent error between the calculated and the experimental values show discrepancies in the meter or the experimental method. This is also evident in their analysis of the experimental data with group contribution models. The Fontan et al. (1981) data does not extrapolate to 1 at the lowest mass fraction of solids while the predictive equation does extrapolate to 1 showing the validity of the equation at low solute concentrations.

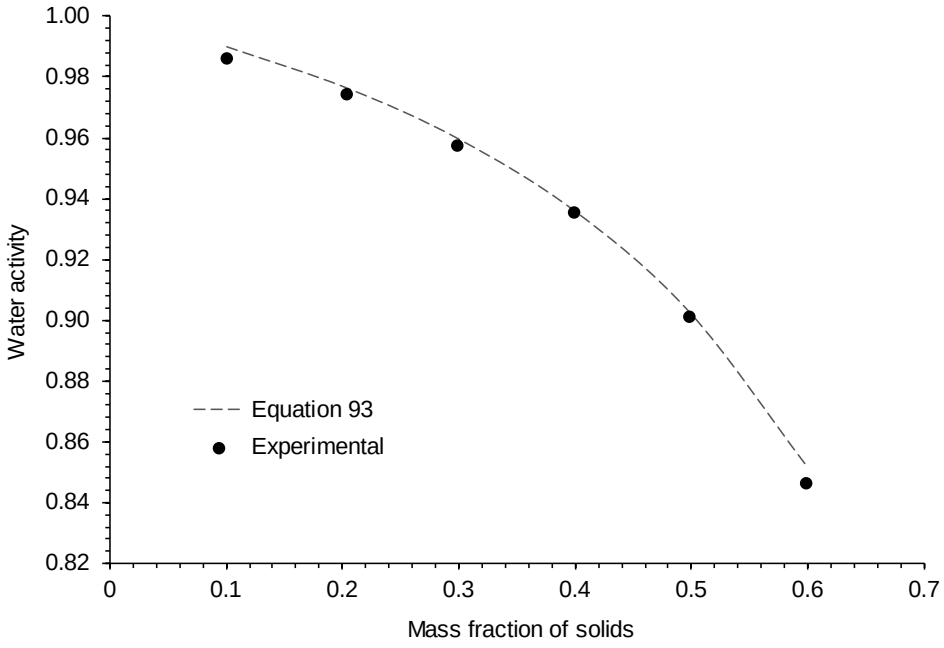


Figure 60 Comparison of water activity data of apple juice containing glucose, fructose and sucrose at 25 ºC (Fontan et al., 1981)

Extending the approach to systems with non-electrolyte sugars and electrolyte (salts and acids), the following observations were made based on analysis of experimental data. The underlying thermodynamic approach of hydration was well suited for sugar systems, so the same approach was applied for electrolytes. Hence, to obtain the hydration number of NaCl from the measured values of water activity, Equation (93) can be rewritten as Equation (94)

$$a_w = \frac{x_w^o - \sum \bar{n}_i x_{s,i}^o - \bar{n}_{NaCl} x_{NaCl}}{\left(1 - \sum \bar{n}_i x_{s,i}^o - \bar{n}_{NaCl} x_{NaCl}\right)} \tag{94}$$

Hence, rearranging the above equation to find the hydration number of NaCl in the food system we get Equation (95).

$$\bar{n}_{NaCl} = \frac{x_w^o - a_w - \sum \bar{n}_i x_{s,i}^o}{x_{NaCl}(1 - a_w)} \tag{95}$$

The concentration for sugars had been expressed as mass fraction, but when electrolytes are involved, mole fraction is more appropriate and thus mole fractions was used for the entire system. Assuming complete dissociation of NaCl, two ion species are formed, but in a system with competition for hydration water, complete dissociation might not happen. However, we consider that the Na^+ and Cl^- ions are equally hydrated. Similarly, for mole fraction calculations for NaCl, it is considered to be twice the contribution. Using Equation (95) the

hydration number of NaCl from the measured water activity data, was calculated to be between -0.045 to 0.03 which is very close to zero. Entirely based on the calculation of hydration number using Equation (90), the understanding we arrive at is that NaCl does not have any hydration water associated. Similarly, it was assumed that the hydration number of malic acid is zero and as the dissociation of malic acid is low, we consider that the contribution towards the mole fraction as a single ion contribution towards mole fraction. The mass fraction of electrolytes in highly concentrated solutions like honey, fruit concentrates, condensed milk, and maple syrup are small, and this approach can be validated. However, a conclusion can be arrived at only when more experimental water activities are measured for systems containing non-electrolyte and electrolyte mixtures.

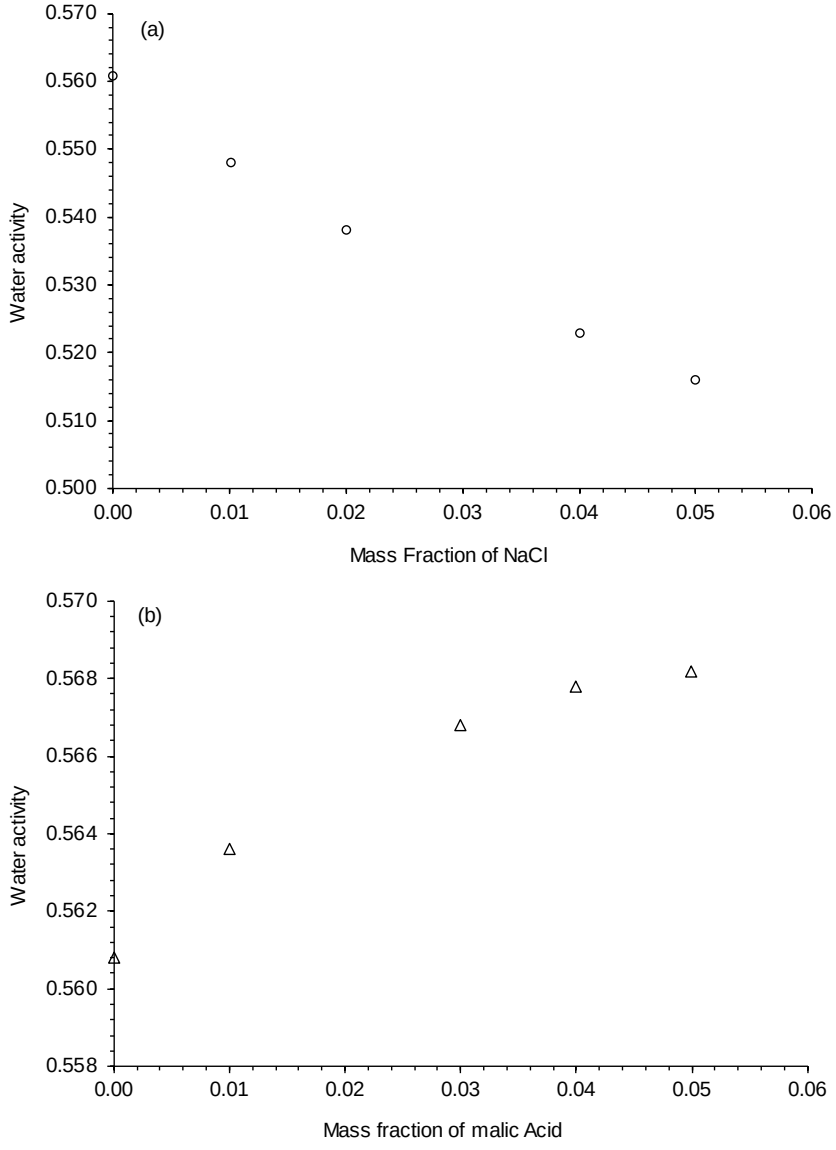


Figure 61 Effect of additives on the water activity of concentrated sugar solutions at 20 ºC. (a) NaCl; (b) malic acid

Figure 61 shows the effect of acid and salt on the water activity measurements of model honey solutions with water mass fraction set to 16% and the solids to 84% with various mass fractions of NaCl and malic acid. Figure 61 (a) shows that addition of NaCl to the solution decreases the water activity of the solution. If the hydration theory as extended to model honey systems is applied here, then due to the amount of sugars in the system, the water activity is reduced. While Figure 61 (b) indicates that the water activity of the solution increases with increase in malic acid mass fraction. The acid increases the water activity of the solutions to a maximum of 0.008 at the highest mass fraction of 5% mass fraction of malic acid. This could be a temporary effect of complete acid dissolution in the system and over time, when the competition for water is high among the sugars and the acid is "separated out", this effect is nulled.

Hence, the water activity of concentrated sugars can be calculated using a zero hydration number for NaCl, $\bar{n}_{NaCl} = 0$. The resultant effect of salt in a complex mixture is the reducing in mole fraction of water that is available for the sugars to compete. Based on the definition of water activity in Equation (35), when the mole fraction of the water is dropped by adding NaCl, the water activity decreases. A similar understanding can be arrived at for malic acid which dissociates into a single ion and the contribution to mole fraction is from the single ion. The hydration number for sugars is calculated at the mole fraction of water in the mixed solution.

Figure 62 shows the water activity of a food system with sugars, acid and salt in terms of mole fraction of water. The water activity prediction using the Equation (93) considers the hydration due to NaCl and malic acid is zero. The prediction and experimental values have a fair to good fit. When using the same approach on a fairly tested system like Rüegg and Blanc (1981) it shows a good fit. Hence, the prediction can be considered valid.

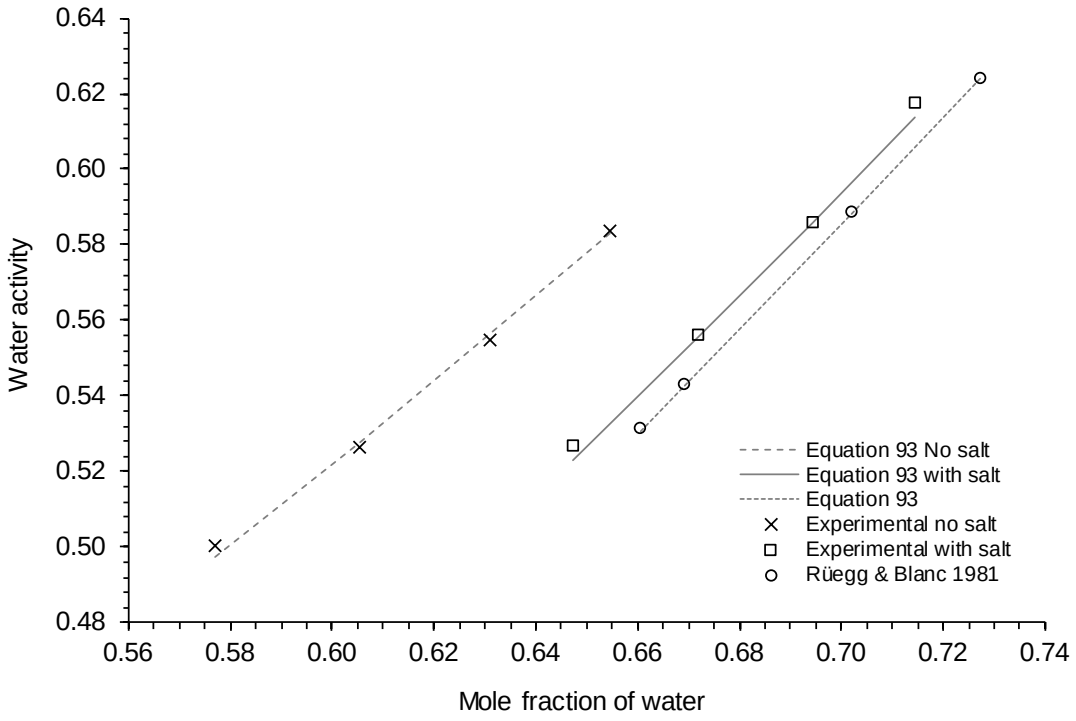


Figure 62 Water activity of a mixture of sugars and salt considering the hydration contribution salt as zero and representing in mole fraction of water. The water mass fraction was varied between 0.155 to 0.2 and the salt mass fractio was varied between 0.04 to 0.0423.

4.6. Mutarotation Studies

Based on the literature review, mutarotation kinetics of sugars are affected by concentration and temperature. Dulski et al. (2016) mentioned that the effect of viscosity on mutarotation is higher than any chemical conformational changes, but this was for molten fructose rather than aqueous systems. However, the effect of solution viscosity on mutarotation has not been investigated much.

4.6.1. NMR

The effect of mutarotation on the crystallization of sugars may be understood by measuring the mutarotation of highly viscous sugar solutions using traditionally applied methods like NMR and optical rotation (Pigman & Isbell, 1968). Le Barc'H et al. (2001) concluded that the preponderance of the β - form of the fructose in solution slowed down the crystal growth rate of α – form of the anomer. In this study we propose a method which can be used to measure the viscosity effect on the mutarotation of sugars at high viscosity like honey.

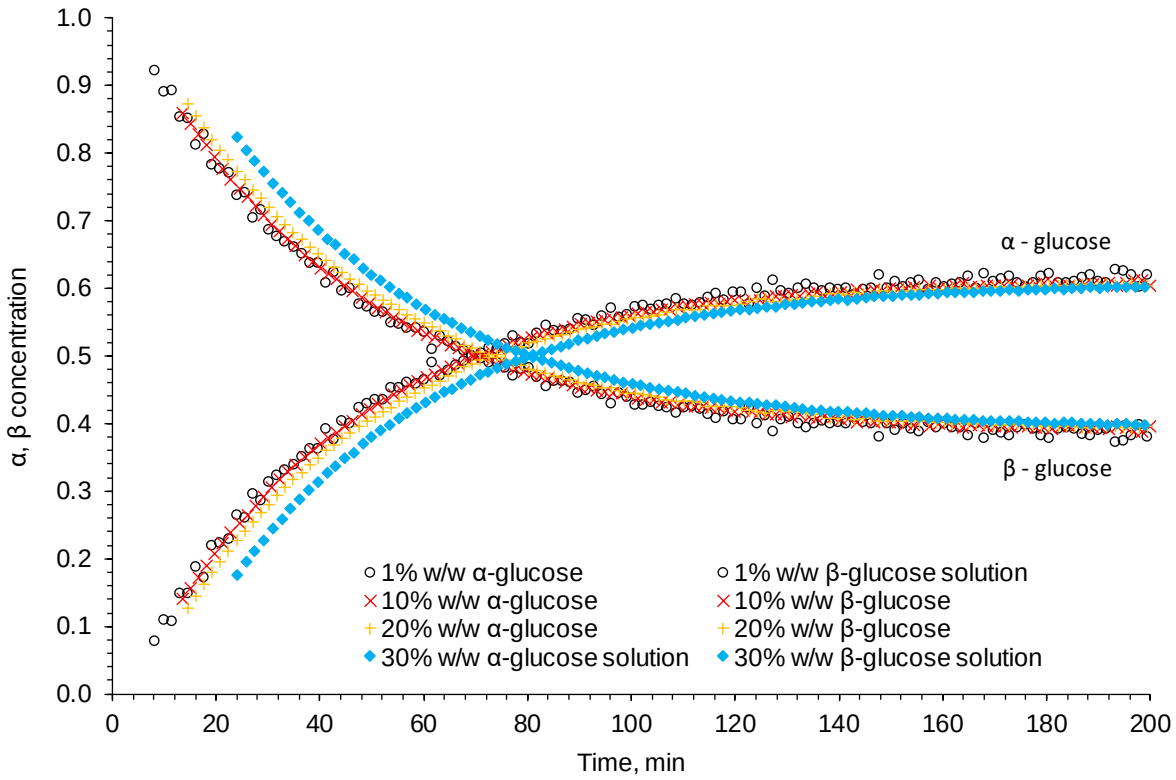


Figure 63 Mutarotation kinetics of glucose solution at different mass fraction and constant temperature of 20 ºC

Figure 63 showing the reaction kinetics of mutarotation for glucose solutions over a range of concentrations and it can be seen that there is slight shift from lower to higher concentration. The experimental data obtained using NMR was then fitted to a standard exponential form $f(x) = c + ae^{-bx}$ and hence the time constants were calculated. However, the effect is not as significant in terms of reaction rate constant as can be seen in the Table 18. Similar effect was seen in the study by Hyvonen et al. (1977), who concluded that the concentration effects remained low while temperature had some effect. Additional test results will be required to confirm this hypothesis.

Table 18 Rheological and kinetic data for glucose mutarotation at 20 ºC

Mass fraction of glucose	Viscosity	Time constant
%	mPa·s	min
1	1	39.08
10	1.3	40.68
20	1.9	40.3
30	3.0	40.3

However, the challenge faced during the mutarotation study is the solubility limit of sugars in solution at a particular temperature. The solubility of glucose in water is considerably low at lower temperatures like 20 ºC. As temperature affects mutarotation, we cannot dissolve the sugars by any other means other than shaking at specified temperatures.

In order to measure the influence of viscosity on the mutarotation of sugars, a viscous solution of sucrose which does not optically rotate was used. A freshly prepared solution of glucose at a concentration of 22.7% by mass with manual shaking at 20 ºC was added to the viscous sucrose solution at 80% solids mass fraction, mixed rigorously at an optimum time and placed in the NMR. Higher glucose concentrations were not possible as the dissolution time at a given temperature would have exceeded the mutarotation time. In these experiments timing began when the first drop of water fell into the glucose solids. The viscosity of the sucrose solution was calculated to be 23.6 Pa·s. Similarly the viscosity of the glucose solution was calculated as 0.0021 Pa·s and that of the combined (glucose + sucrose) solution was calculated as 0.017 Pa·s.

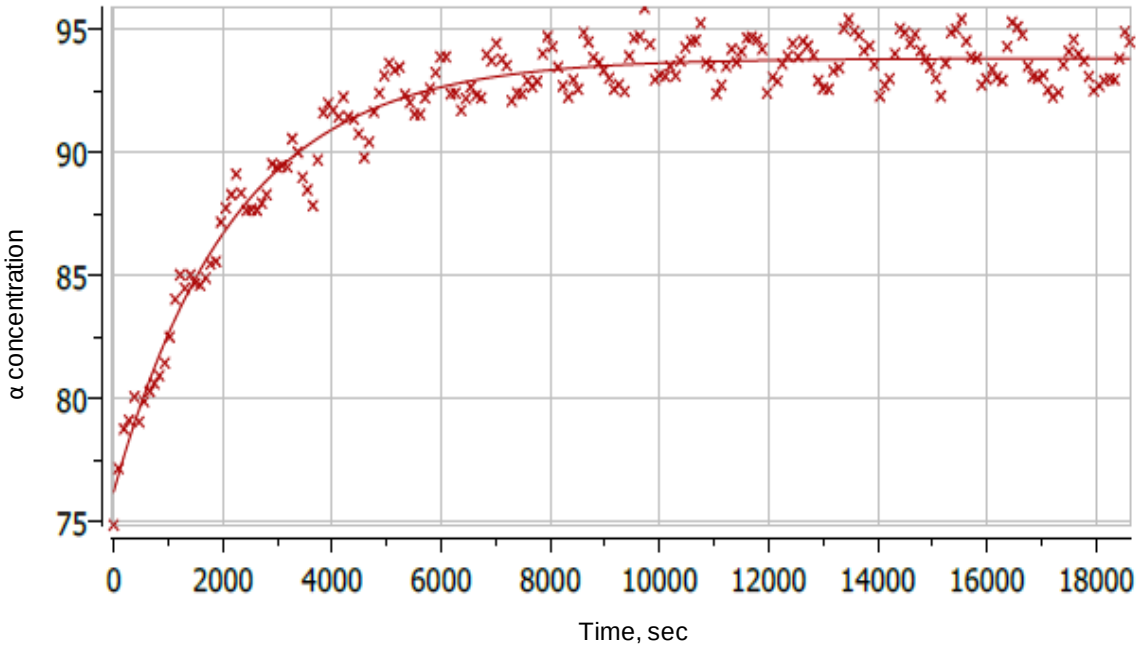


Figure 64 NMR data of 22.7% glucose solution in a 80% sucrose solution at 20 ºC

Figure 64 shows the concentration of the beta glucose increasing in solution over time in seconds due to mutarotation. The time constant obtained by fitting an exponential through the experimental NMR data in Figure 64 was 40.3 min. This value is closer to the observed values

of time constants for binary solutions of glucose at the same temperature. The difference in the values may be attributed to increase in experimental noise caused by lower concentration of glucose in the sample after dilution by sucrose. This indicates that the viscosity of the solution may not have any effect on the mutarotation of a sugar. In a food system with more than one sugar, the mutarotation of one of the sugars is not affected by viscosity of the solution. More data should be created in mixtures to confirm the conclusion on this effect.

4.6.2. Optical Rotation

Kraus and Nyvlt (1994) used a simple model for the optical rotation of anhydrous glucose to measure mutarotation kinetics. A similar approach is proposed in this study. The rate of change of rotation can be represented as a first order reaction

$$\frac{dC}{dt} = -k\,C^n \tag{96}$$

Here C is the concentration (as measured by rotation), k is a constant and n is normally considered to be 1. Hence, for the α, β anomers,

$$\frac{dC_\alpha}{dt} = -k_1 C_\alpha + k_2 C_\alpha \tag{97}$$

$$C_\alpha + C_\beta = C_{total} \tag{1}$$

Then, the rotation angle α_r is assumed to be a linear function of anomer concentration

$$\alpha_r = b_\alpha C_\alpha + b_\beta C_\beta \tag{1}$$

The two rate constants can be related using an equilibrium constant

$$K_m = k_1/k_2 \tag{1}$$

Kraus and Nyvlt (1994) gave a value for K_m of 1.72 which they stated was almost independent of temperature. When these equations are combined they give a first order exponential response. The time constant for the data in Figure 65 was found to be 48.2 minutes with an adequate fit. This was higher than values around 40 minutes obtained using NMR (Table 18).

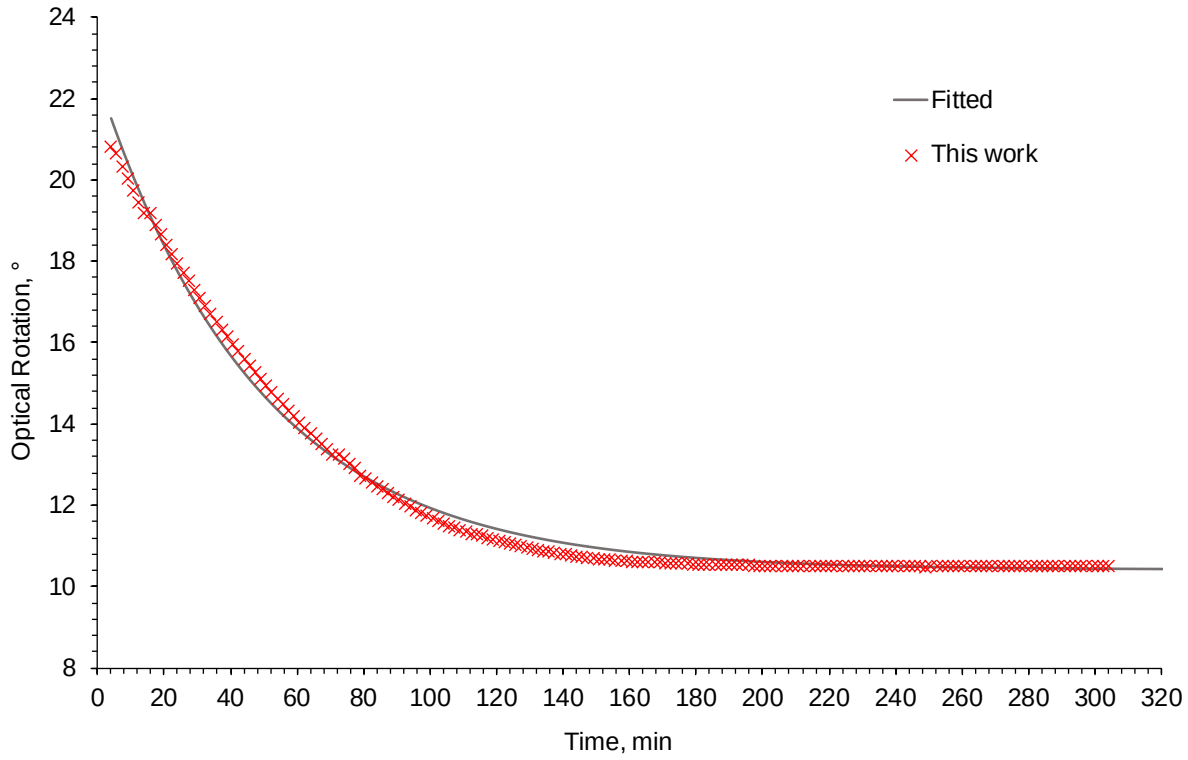


Figure 65 Optical rotation of 22.7% glucose solution at 20 ºC

The NMR and optical rotation measurements of mutarotation lead to the conclusion that the rate of mutarotation is not strongly affected by sugar concentration or viscosity. Referring to Figure 15, this implies that very little movement of the molecule is required for mutarotation to occur. Hence it is unlikely to be a rate determining step during crystallisation from concentrated sugar solutions, but instead the diffusion of molecules towards crystals is likely to be limiting.

4.7. Crystallization

In this section, the effect of indicators used to understand and further predict the crystallization of sugars in concentrated sugar systems is investigated. Some results are presented but they show the need for further refinement of methods to achieve repeatable results and hence no error calculations could be performed. The consistency between consecutive points indicate an uncertainties of$\pm$0.003. Measurements obtained in solutions containing crystals were much less consistent than for liquid only systems. The effect of crystallization is measured using water activity measurements. In the first set of experiments as described in Table 9 under

Section 3.2.2.7., the fructose/glucose (F/G) ratio is varied with and without seeding and additionally stirred or not stirred. In the entire set of experiments, the total mass fraction of water always maintained at 17%.

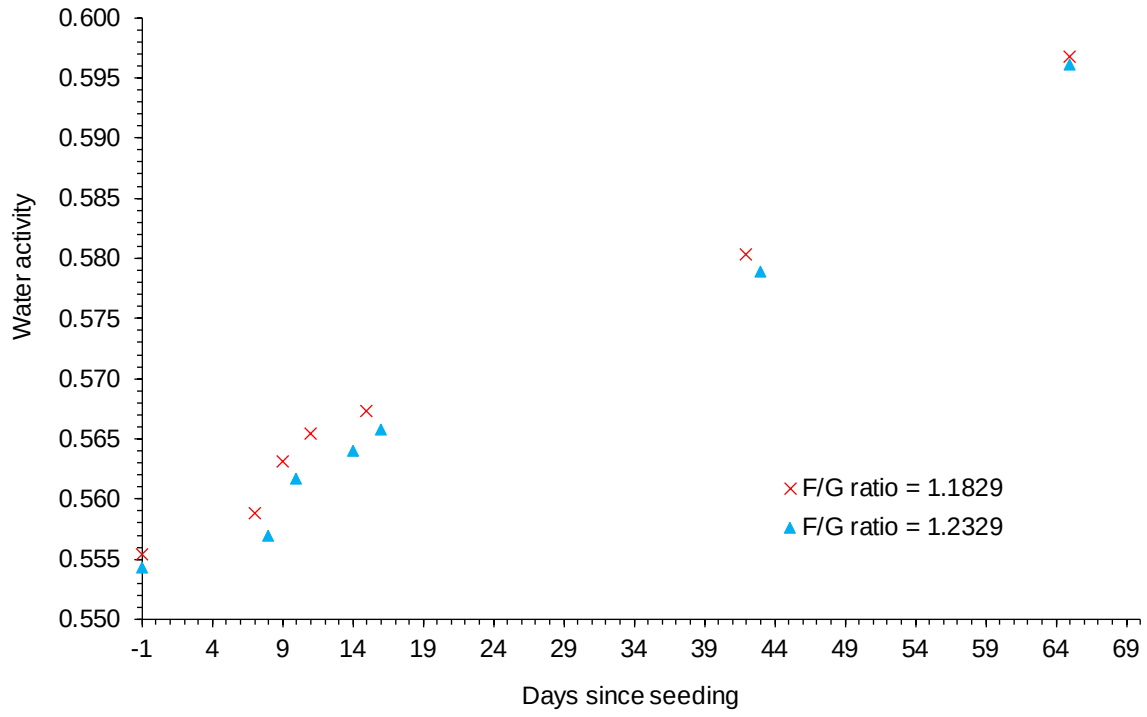


Figure 66 Effect of F/G ratio on the water activity of the model food system at 20 °C and 1% seeding

Figure 66 shows the variation in water activity of a model honey solution with different F/G mass ratios of 1.1829 and 1.2329 at constant water mass fraction of 17%. The higher the F/G ratio, the lower the water activities measured and this indicates that the fructose in the solution attracts more water than a solution with less fructose.

Figure 67 shows the effect of seeding in solutions with the F/G ratio kept constant at 1.2329 as per Table 8. The solution with 1% seeding, had high crystallization effects. After 5 days the solution appeared to be completely crystallized and hence the water activity measurements are ambiguous. This was a persistent problem with sugar solutions with a high solid fraction of 83%.

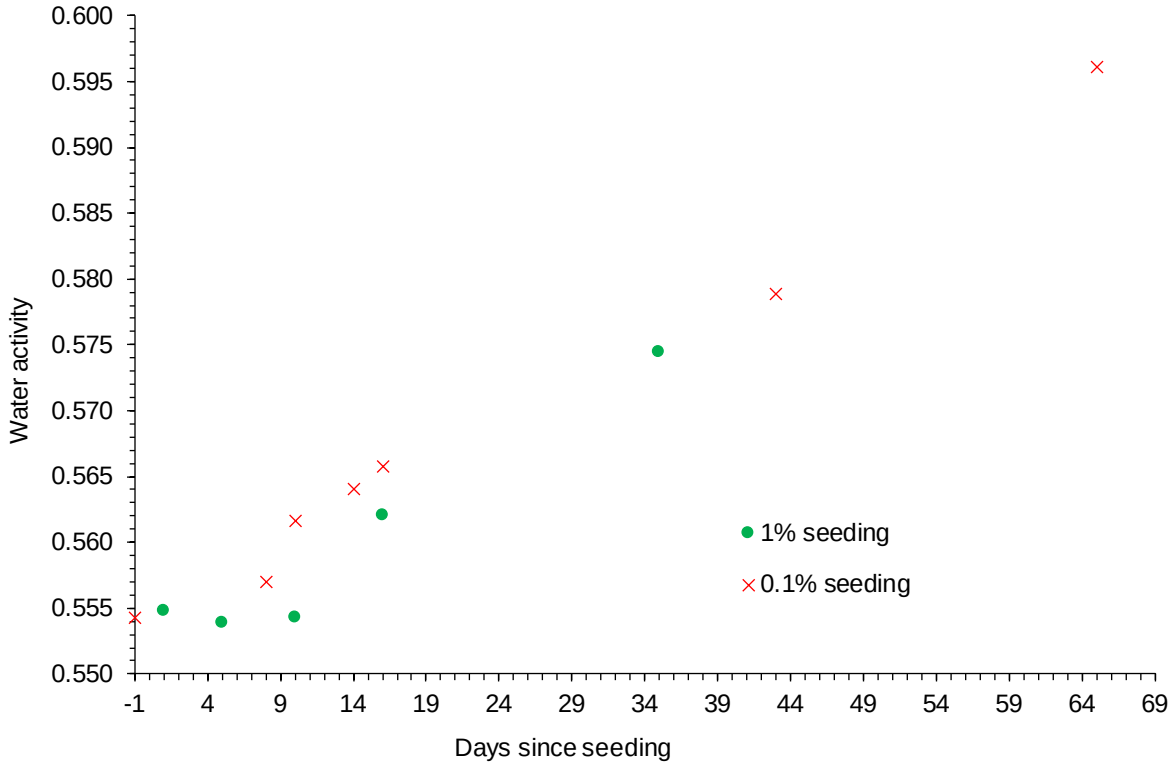


Figure 67 Effect of seeding on the water activity of the model food system at 20 ºC and F/G = 1.2329

The effect of stirring was studied in a solution by choosing the best seeding mass fraction of 0.1% and F/G ratio of 1.2326 (close to 1.2329). The solutions were stirred at 135 rpm at 20 ºC throughout the course of this experiment. The experiment was not continued after 9 days due to complete crystallization of the solution. However, the effect of stirring seems to be a linear relation with the number of days as shown in Figure 68 and this seems a better option as this might help remove any air bubbles in the system.

In the next set of experiment, the effect of water content on the water activity measurements was observed. Invert sugar mixtures were prepared with sugar mass fraction from 81% to 84%. These mass fraction were well in the high viscosity range between 6.9 and 30.3 Pa·s as calculated. The trend observed in this experiment was as expected in Figure 69. The water activity of the solutions with higher water content were higher and the ones with the lower water content were lower.

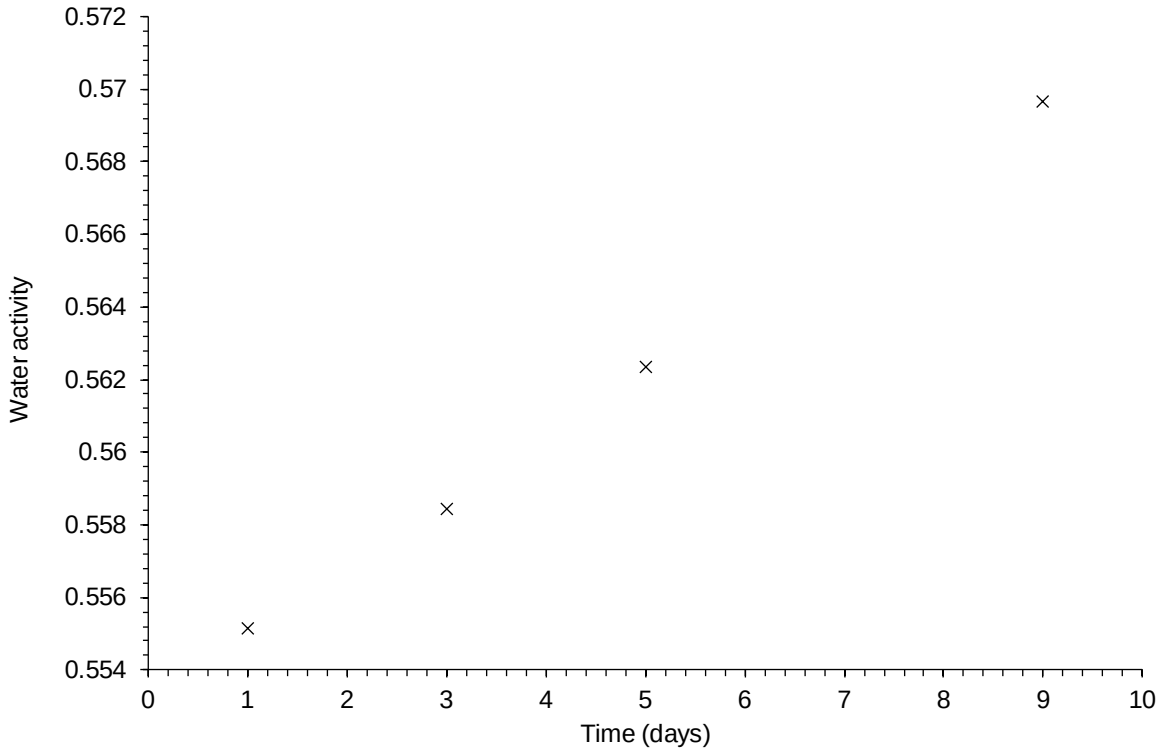


Figure 68 Effect of stirring on water activity of mixture of sugars at 0.1% seeding, F/G=1.2326 at 20 ºC

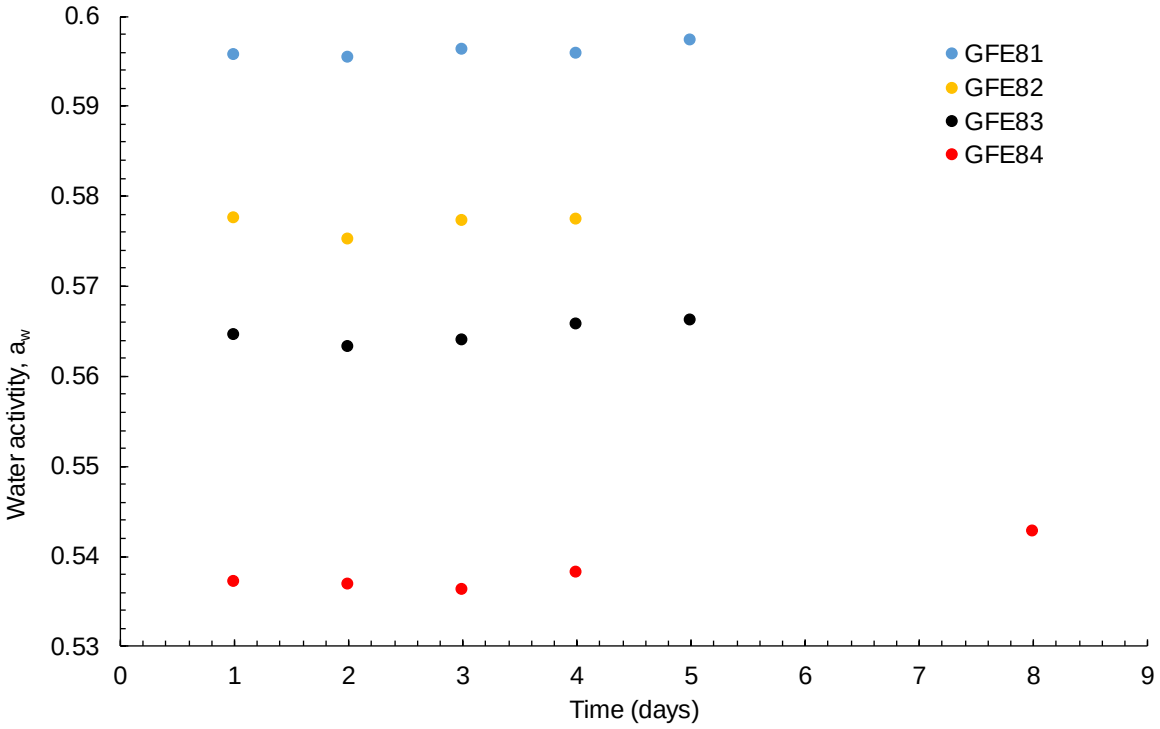


Figure 69 Effect of water content on the water activity of invert sugar mixtures at 20 ºC.

As per Table 10 under Section 3.2.2.7., an attempt was made to understand crystallization speeds in hours at different seeding mass fractions keeping the F/G at 1.2329, S/G at 0.0411 and total water mass fraction at 17%. Figure 70, shows that increasing the seed crystal fraction increases the crystallization of sugars. It was observed microscopically that crystallization of sugar starts as early as 10 hours from the time of seed addition. This showed the need for more measurements within the first few hours as well as measurements over many days.

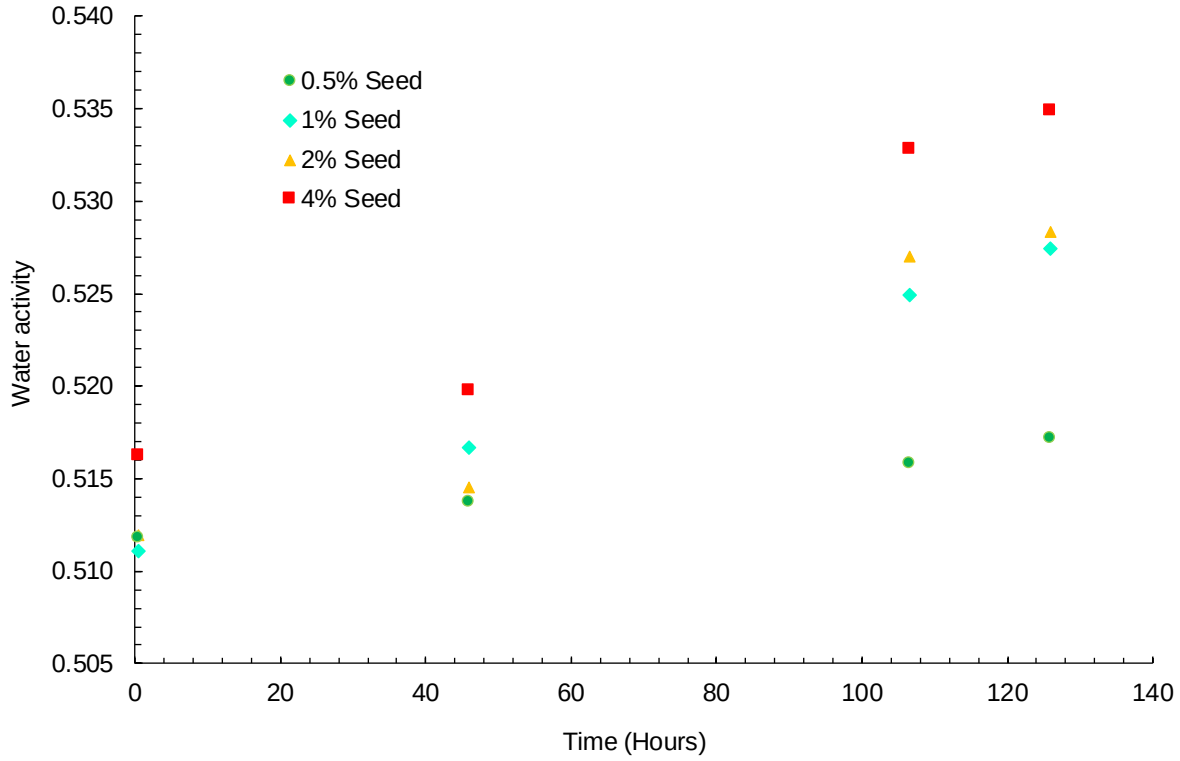


Figure 70 Crystallization rate over time in hours at various seeding mass fractions at 20 ºC

Figure 71 shows a curious result with model honey solution containing 16% water, 5% malic acid and 79% invert sugar. The water activity of the solution decreased over time. The crystals in the solution were not glucose crystals on visual examination. When glucose crystallizes, the water activity of the solution increases due to the release of water molecules. In this case, the water activity increased over time. This suggests the competition of water in a system with 16% water, 5% malic acid and 79% invert sugar, is mainly between the sugars and not the electrolyte. The malic acid in the system slowly "separated out" over time. This effect was observed in the solution. Even after 300 days, the sugar remains in liquid form (dissolved in water) as in solution while the acid settled at the bottle of the container. Though this observation is made on visual observation over time, Figure 71 also suggests similar tendencies. This needs

to be validated over a range of mass fractions of acid, salt and sugars as this might also be a solubility effect.

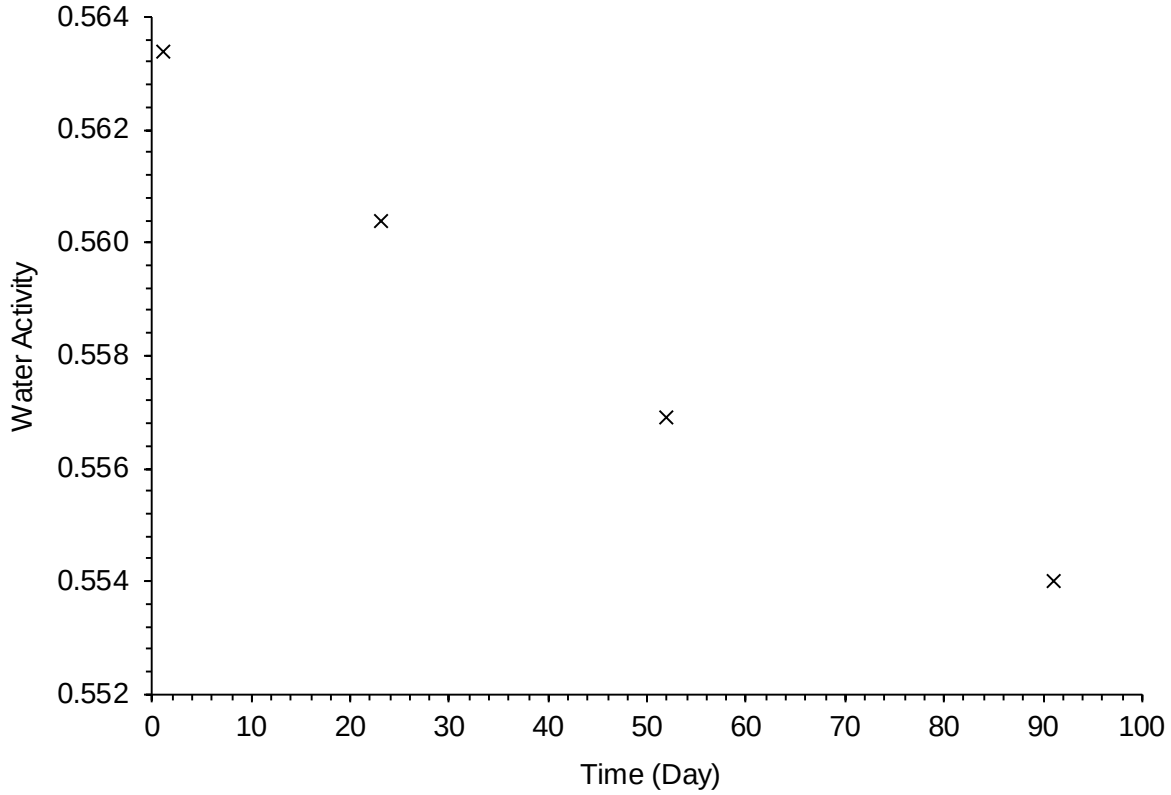


Figure 71 Water activity measurement for a model honey solution with 84% solids. Malic acid contributes 5% of the total solids in the solution with 16% water mass fraction.

In conclusion, when an approach is used as an indicator to measure crystallization of sugar in complex solutions, a more thermodynamic approach needs to be taken. All the indicators in Table 6 are purely empirical and these may be used by bee keepers, but might be less valuable for controlling processes at industrial scale. Figure 72 shows some of the factors that may affect crystallization of sugars in a complex sugar system. These factors have some basis as discussed in Section 2.5. There are many interactions that are not shown in detail.

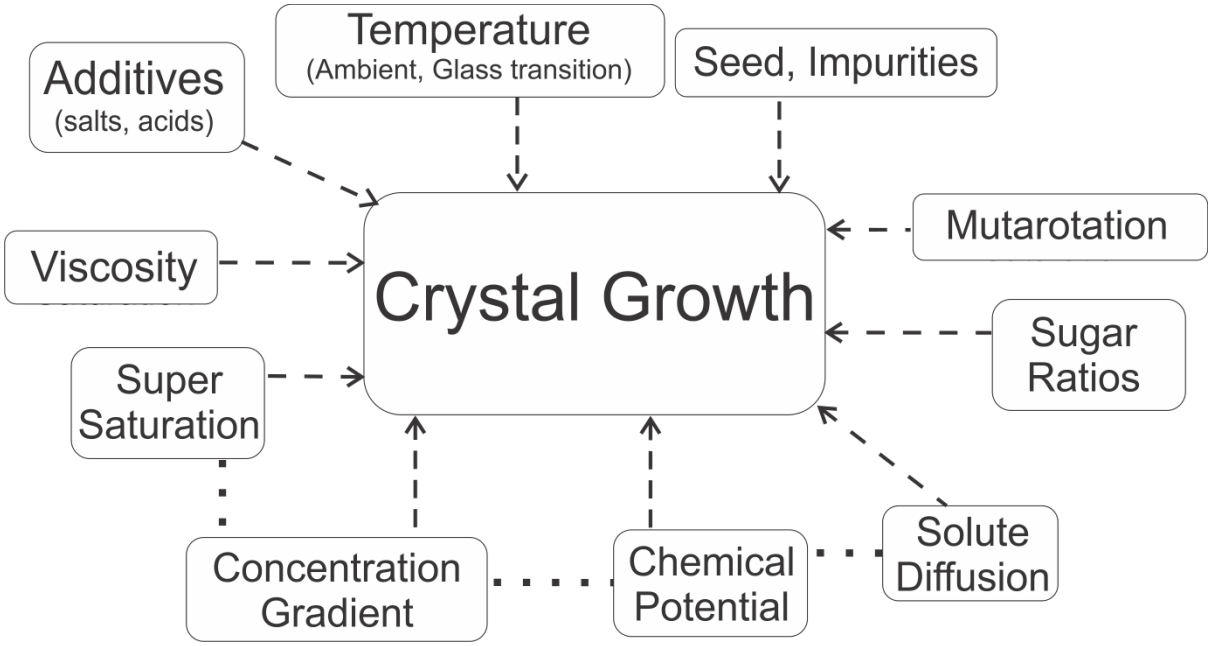


Figure 72 Factors that influence crystal growth in a typical food system. The dashed lines represent the link that is existing between the parameters.

It is evident from this work that the amount of water in such food systems play a larger role in the crystallization process. Results indicate that viscosity does not affect the mutarotation of sugars and hence can be concluded that it would not affect crystallization of sugars. Seeds definitely induce crystal growth as evident from the experiments. It can be said that super saturation, concentration gradient, solute diffusion, chemical potential may affect crystal growth. However, based on experiments the same cannot be said about sugar ratios. Sugar ratios may be used as indicators to forecast the crystal growth but this requires further studies.

4.8. A Model System with Property Predictions

In a model system with sugars the following properties can be predicted using the proposed equations and models. Considering a food system with glucose, fructose and sucrose, properties like density, viscosity, conductivity and water activity can be predicted with considerable confidence. Most of the properties are based on the amount of water present in the system and not on the total sugars in them. Hence, all food systems with sugars and traces of acids and salts can be approached on this basis. Figure 73 shows the properties predicted and some with measured values.

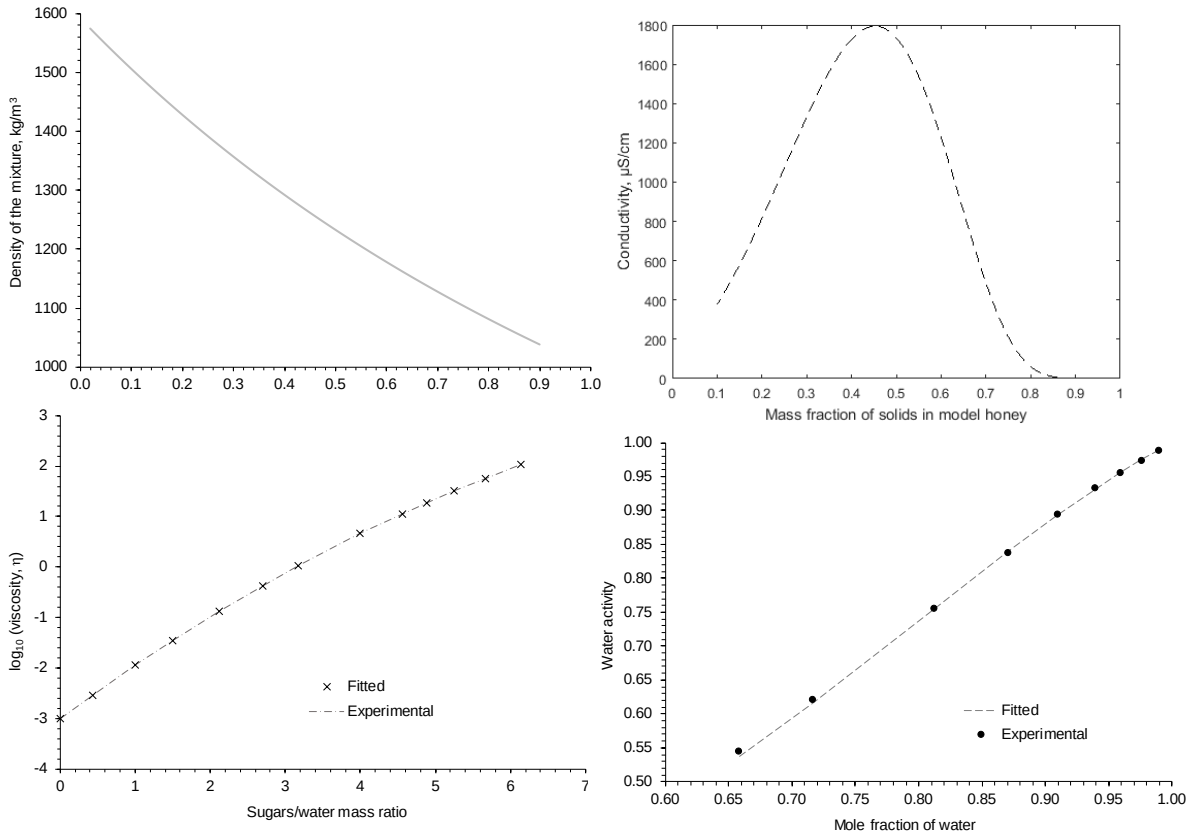


Figure 73 Predicted properties of a model food system with sugars at 20 ºC

The aim of this study was to accumulate and extend experimental data, apply better correlations to existing data, understand the influence of water on each property and extend relationships to other food systems. Most of the aim was achieved to the best over a range of concentrations, and temperature.

5 CONCLUSIONS

Densities of binary sugars in the literature were analysed using the theoretical equation and this principal was extended to complex sugar systems with multiple sugars. The density of binary glucose, fructose and sucrose solutions can be predicted with the theoretical equation with maximum errors as shown in Table 19 in comparison to the most appropriate literature data excluding outliers after careful analysis. Similarly, when extended to sugar mixtures, the predicted density of invert sugar and model honey solution the maximum error are also shown in Table 19.

Table 19 Density error from the predicted equation at 20 ºC

Solution	Error (kg/m^3)
Glucose	0.003
Fructose	0.011
Sucrose	0.82
Invert sugar	0.0003
Model honey	4.07

Viscosity of sugar solutions were measured and compared with existing literature data and these showed good agreement with the predicted viscosities. Table 20 below shows the maximum relative errors for binary glucose, fructose and sucrose solution along with invert sugar solutions. More experimental data points are required for a model system with both electrolytes and non-electrolytes to calculate the errors.

Table 20 Viscosity errors from the predicted equation at 20 ºC

Solution	Mass fraction range (%)	Max. rel error (%)
Glucose	0-60	3.2
Fructose	0-70	3.7
Sucrose	0-70	3.5
Invert sugar	65-84	0.24

Refractive index was confirmed to be almost linear with volume fraction and linear combinations of volume fraction for sugar mixtures give predictions accurate to $\pm$ 0.04%. The effect of salts on the viscosity was negligible and that of 5% malic acid reduced the refractive index by only 0.0005. The refractive index of binary sugar solutions was best predicted using the least squares fit proposed by Reis et al. (2010). However, for the more concentrated model honey solutions, the model that considers ideal thermodynamic mixing is best suited. The models proposed by Reis et al., (2010) are sensitive to the value of the pure liquid sugar

refractive index. Methods of determining these values were investigated and it was found that appropriate values could be determined by using Solver in Excel to minimise the sum of the absolute error between the calculated values and refractive index data from Weast (1978).

In case of electrical conductivity of binary sugar solutions, it can be concluded that the contribution is almost entirely based on the salts present in the sugars during manufacture. The contribution from the dissociation of sugars is near zero and hence it was concluded that the electrical conductivity measurements of sugar solutions can be directly related to the salt (ash) content in the system. The conductivity was found to be strongly influenced by the solution viscosity which reduced ion mobility. The proposed model is well suited to predict the electrical conductivities of binary sugar solutions and model honey solutions. The model also predicts the value of electrical conductivity of a model honey system as 0.8 mS/cm as described by Codex Standard 2001 using a 20 g of dry honey in 100 mL of water. This again validates the model.

The extension of a similar model to food systems like milk, sodium carboxy methyl cellulose (NaCMC) and whey protein isolate (WPI) solutions indicate that the use of diffusion viscosity suits better compared to bulk viscosity. Thus, it was confirmed that in systems where the viscosity is dominated by long chain molecules such as proteins, cellulose and carbohydrates, the diffusion viscosity affects the electrical conductivity. The fundamental approach used in the measurement of electrical conductivity has helped in understanding viscosity effects of ternary sugar solutions. Hence, the viscosity effects can be incorporated and implemented in physical properties like diffusivity and better models can be developed.

Water activity of sucrose solutions were well analysed in literature using Scatchard's approach (Starzak & Mathlouthi, 2002, 2006; Starzak & Peacock, 1997; Starzak et al., 2000). A similar hydration theory approach was applied to glucose, fructose, maltose and glycerol, and data from literature and experiments were analysed on the basis of hydration water. The sensitivity of water activity measurements in hydration number calculation is illustrated in Figure 55. In a sugar solutions with glucose, fructose, sucrose, NaCl and malic acid, the hydration of electrolytes was analysed. It was found that the hydration of malic acid and NaCl was near zero and also noted that the only effect these electrolytes had were by lowering the mole fraction of water in the system. Hence when a food system with multiple sugars and these electrolytes are present, the data is best represented by mole fraction. Further measurements and data analysis

is required to validate the conclusion that NaCl and malic acid does not have any contribution to hydration.

Physical property data for binary to complex sugar solutions like honey were consolidated and complied. This consolidated data can be used for engineering design and process development. The measured physical properties of modelled honey solutions were compared to those of natural honey. Variability in the moisture content and other sugars in honey can be explained with the obtained data. The influence of water in physical properties like viscosity, RI and water activity has been discussed and their effects on honey and its solutions are summarized. Indications of near-zero effect on refractive index due to crystallization or longer storage of solutions has been observed in this research and can be stated that refractive index can be used as a tool to confirm concentration of solutes in a food system. Mathematical correlations that can be applied to binary, ternary and complex sugar systems have been derived and summarized. The correlations are fitted to existing data as well as newer data. New models are developed which has improved the prediction capabilities from the existing models. These calculated errors were well within the range of experimental errors. This indicates that the proposed equations are useful for most engineering applications.

6 FUTURE WORK

Recommendations for future work are made below.

- Development of theoretical equations and refinement of existing equations for different physical properties are essential. For instance, the effects of salts and acids needs to be better understood to focus on working towards better predictive equations.
- Development of the technique to model solutions like honey has made way to approach problems like crystallization in a holistic sense of incorporating all the physical properties as discussed in the results section. Hence, crystallization of sugars in such a complex sugar system can be modelled incorporating all the proposed equations and individual models.
- As the accuracy in water activity measurements have increased through this work, use of hydration number for complex sugar systems can be used as a tool for other food systems containing sugars. The hydration approach to acid and salts needs to be further tested to get better conclusions.
- On the basis of this work, it is recommended that sucrose solutions can be used as calibration standards for water activity instrument. It was observed to be a clear indicator of any deviations. Solutions can be prepared with very good accuracy over a wide range of concentrations and can be verified using refractive index.
- The approach towards using water concentration as a basis for all measured physical property in this study has provided a standard approach of study in food systems with sugars. This approach would help enumerate the understanding of food systems better. For mixtures, water activity was best based on water concentration rather than solute concentration. Also, viscosity was based on the ratio of solutes to water which showed a linear relationship at lower concentrations. These approaches can be extended to other food systems.
- The contribution of additives like salts and acids towards the measured physical properties in this work has provided a basis for a food system like honey. Hence, this approach can be extended by studying more systems with various acid and salts.
- Accuracy has been the primary focus on physical property measurements and the work has focused on developments of methods for this purpose. Hence, these developed methods can be utilized for research in future.

- Solubility measurements on sugar systems were not completely studied as approached by Kelly (1954), need to be revisited to get much greater understanding of the effect of solubility on crystallization. The influence of temperature and composition on solubility can be related to sugar activities. Further studies on solubility needs to be performed and solubility limits can be determined.
- The approach towards crystallization of these ternary solutions that are close to honey should be changed in the future and all existing indicators like fructose/glucose, sucrose/glucose should be tested. This research work recommends the use of sugar/water ratios compared to sugar ratios like fructose/water and glucose/water as potential indicators for crystallization prediction.

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APPENDICES

APPENDIX A Method to determine water activity value

Finalising method to collect data for water activity of a sample based on preliminary experiments with concentrated sugar solutions. As it can be seen that the water activity of the solution equilibrates and plateaus to a value within ± 0.0005. Then the values can be fitted by eye and the water activity value is noted.

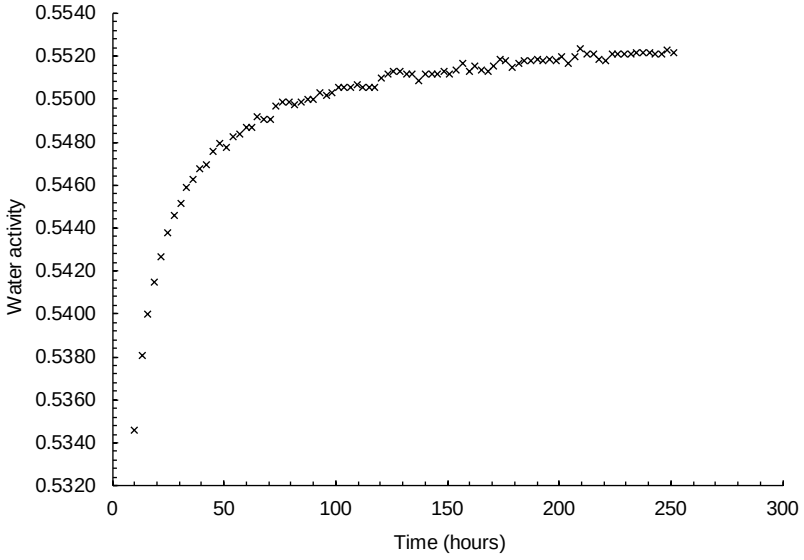

Figure 74 Water activity determining method

Within about 80 minutes the water activity was within the manufacturer's specified accuracy of ± 0.003, but after 350 minutes the measurement was stable to ± 0.0004.

METHOD OF MEASURING WATER ACTIVITY USING THE INSTRUMENT

The method used to test the water activity of model honey solutions for the current experiment are detailed below:

1. Turn on the water activity meter using the power switch located on the back of the equipment and leave for approximately 15 minutes to equilibrate. For first time use, any stored data should be reviewed and cleared.
2. Set the measurement temperature to 20 ˚C using the control buttons beneath the display. Find a clean, plastic sample cup and ensure it is completely dry before use. Using a measuring spoon, take approximately half a teaspoon of model honey solution and

carefully pour into the middle of the sample cup. For a maximum level of sample to add, refer to the AquaLab Quick Start Manual (reference).

3. Carefully rotate sample cup to ensure the bottom surface is completely covered by solution, without air bubbles.
4. Confirm sample cup is completely clean on outside before moving the measurement lever to the right-hand, ‘Open’ position, lifting the chamber lid and placing cup in the sample holder.
5. Seal sample in the holder by placing down the chamber lid and moving the measurement lever to the left-hand, ‘Read’ position.
6. After several minutes, the equipment will be equilibrated to the set temperature and measurements of water activity will be taken automatically by the meter every two minutes, signalled by a beep noise.
7. After a total of four hours, move the measurement lever to the right-hand, ‘Open’ position, lift the chamber lid and remove sample cup from the holder.
8. Rinse sample cup with DI water and dry with a delicate wipe (kimwipe). Repeat a further two times and place the completely dry sample cup upside-down on a kimwipe for later measurements.
9. Results from the readings including temperature, water activity and the date and time of each measurement are stored in the meter. Collect and record measurement data using the associated computer in the SP lab and ensure the computer has internet access enabled when doing so.
10. If no further measurements are to be made, turn off both the water activity meter and corresponding computer.

Prior to analysis of model honey solutions, the water activity meter was calibrated using a sample of DI water and the operation steps listed above were followed. Results of the calibration of water presented a water activity of 1.0000 ± 0.0002, which was concurrent with expectations (Te Aika, 2016). Hence, no correction calculations were required for the final results of model honey measurements.

APPENDIX B Water activity meter calibration

Water activity standards were used to calibrate the water activity meter prior to a series of tests conducted and water was used always before performing a series of tests. Please find the calibration data in the Table 21 below. Figure 75 illustrate the variation in water activity measurements over time.

Table 21 Water activity meter calibration using standards from Decagon, USA with descending order of water activity

Sample	Conc, mol/kg	Water activity of standard	Measured water activity
Water		1	0.9995 ± 0.0002
Potassium Chloride	0.5	0.984	0.9840 ± 0.0003
Sodium Chloride	2.33	0.92	0.9202 ± 0.0001
Sodium Chloride	6	0.76	0.7602 ± 0.0001
Lithium Chloride	8.57	0.5	0.4993 ± 0.0001

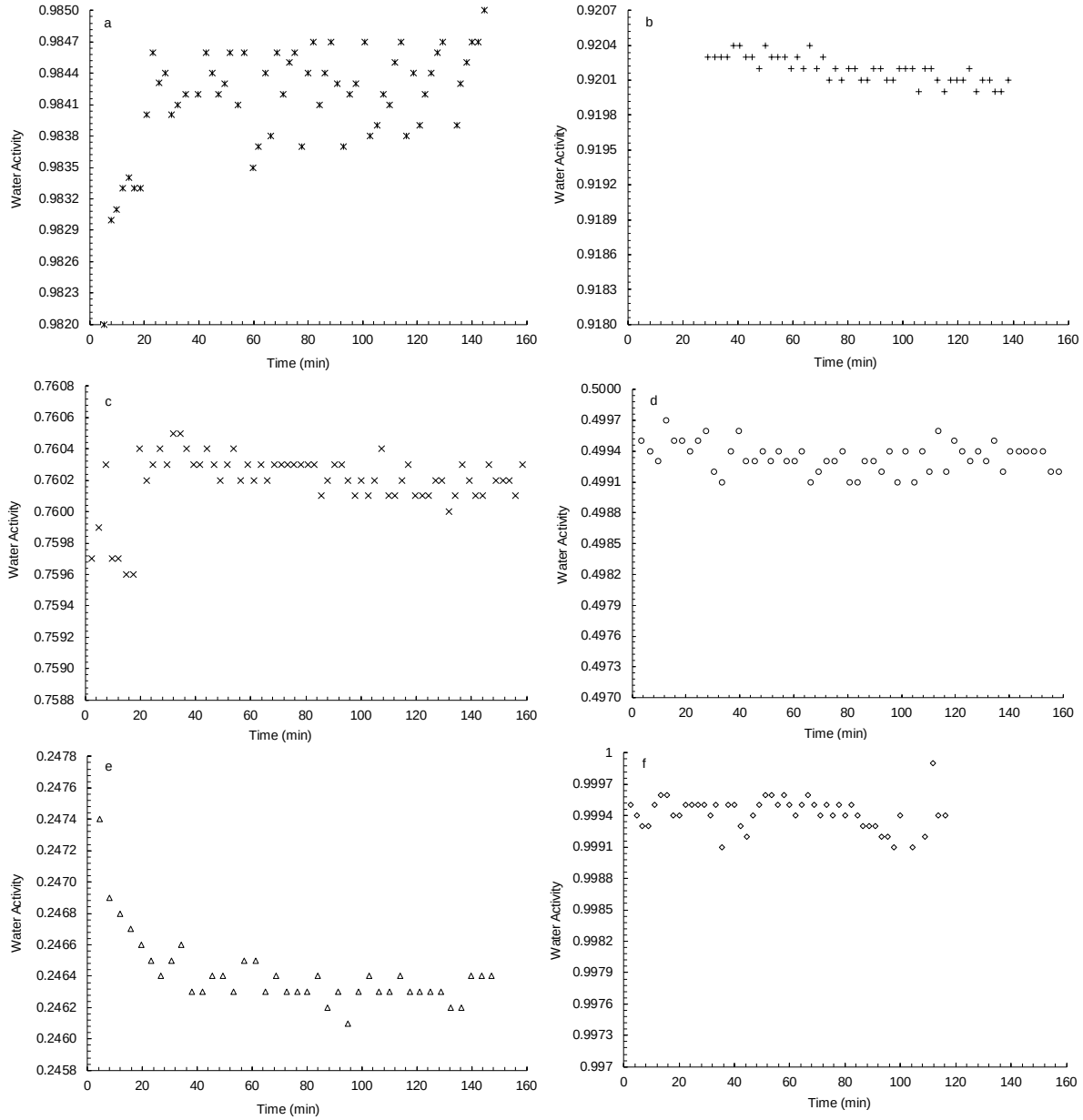

Figure 75 Water activity calibration using standards supplied by Decagon, USA and water. Starting from left to right from top: (a) Potassium Chloride (0.5 kg/mol), (b) Sodium Chloride (2.33 kg/mol), (c) Sodium Chloride (6 kg/mol), (d), Lithium Chloride (8.57 kg/mol), (e) Lithium Chloride (13.41 kg/mol), (f) Water.

APPENDIX C Conductivity set-up calibration

The calibration of the conductivity cells were carried out using standard solutions of KCl. All standard solutions were prepared based on calculated weights of KCl and milli-Q water. The conductivities of the solutions thus prepared were measured and compared with the standard literature values as in Weast (1978) as shown in Table 22 below. Table 23 shows the calibration data of the prepared standard solutions of KCl. Upon calibration, the conductivity measurements are calculated where the phase is zero or near zero. Figure 76 shows the very good fit between experimental and literature data.

Table 22 Conductivities of KCl standard solutions at 20 ºC ((Weast, 1978))

Mass Fraction (%)	kg KCl/ kg solution	Solution concentration w/w KCl/water	Conductivity (σ) in (µS/cm)
0.05	0.005	0.0050	8200
1	0.01	0.0101	15700
2	0.02	0.0204	29500
4	0.04	0.0417	57600
7	0.07	0.0753	100000
9	0.09	0.0989	129000
10	0.1	0.1111	143000
12	0.12	0.1364	172000
15	0.15	0.1765	208000

Table 23 Calibration of the conductivity cells using KCl standards.

Mass Fraction (%)	CRC kg KCl/kg soln	kg KCl/kg soln	Temperature (°C)	Frequency (Hz)	Resistance (Ω)	Phase Angle	Measured Conductivity (µS/cm)
10	0.1	0.1	20	40000	6.7362	-0.3	128411
1	0.01	0.01	20	100000	55.117	-0.1	15694
0.1	0.001	0.001	20	10000	497.16	-0.8	1740
0.01	0.0001	0.0001	20	1400	4673.4	-0.6	185
0.001	0.00001	0.00001	20	100	43558	-0.4	20
0.0001	0.000001	0.000001	20	100	279340	-1.5	3.1
0.00001	0.0000001	0.0000001	20	100	640570	-3.3	1.4
0.000001	0.00000001	0.00000001	20	100	527140	-2.7	1.6

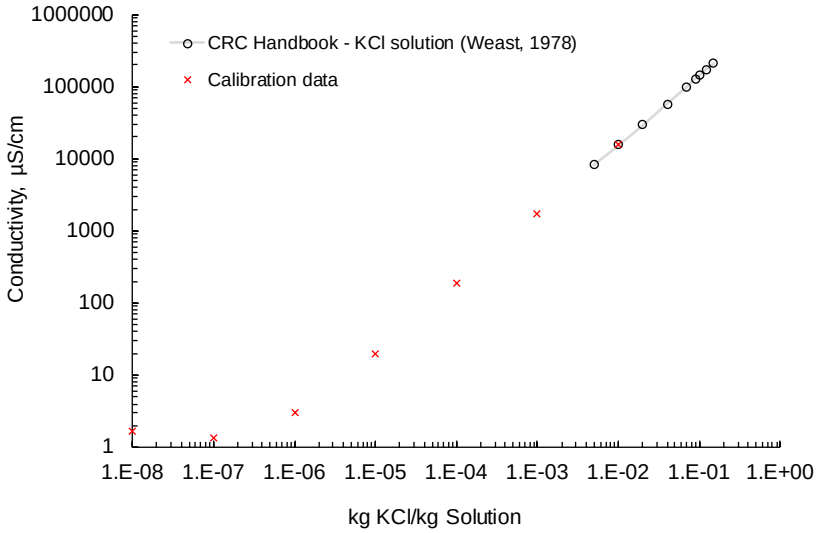

Figure 76 Log-log graph showing the close agreement the conductivities of KCl solutions with the CRC data (Weast 1978)

APPENDIX D Viscometer calibration

The RotoVisco was calibrated using standard N1000 calibration sample for a MV1 spindle and cup arrangement. The measured stress was corrected from a linear fit obtained from the data of the calibration liquid as shown in Figure 77. This approach was followed each time a measurement set was to be obtained. An error of 0.002 Pa·s was obtained in the last calibration done on 18th July 2017.

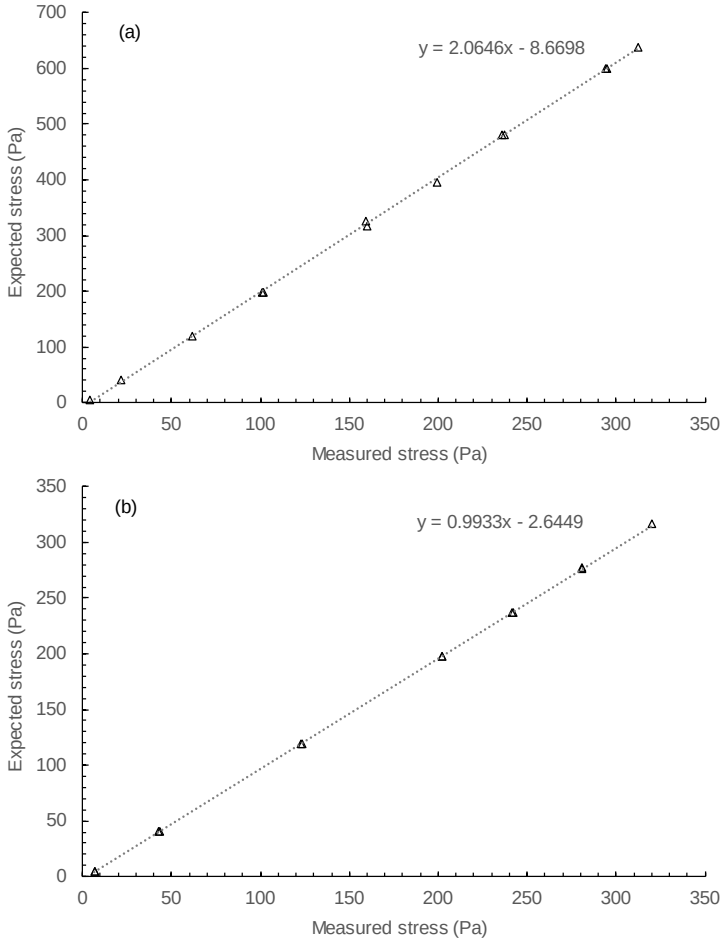

Figure 77 Calibration of the viscometer using standard calibration liquid N100 at 20 ºC. (a) using old N1000 sample (10th July 2017); (b) using newer sample (18th July 2017).

APPENDIX E Refractive Index calibration

The refractive index can be used to confirm the sugar concentrations for model honey solutions. Refractive index measurements were done using Abbe NAR-3T Refractometer.

CALIBRATION

1. Rotate prism latch down and rotate top prism to open position. Using a dropper, place several drops of DI water on sample prism. Close top prisms gently. Rotate sample prism latch up to secure prism in place.
2. Look into eye piece. If the colour compensator field is all light or all dark adjust the measurement knob to read 1.3330 as the refractive index in the measurement window. Focus can be achieved in the measurement field by rotating the eye piece.
3. Adjust the colour compensator knob until the colour compensator field boundary is in focus and showing the least red or blue boundary coloration.
4. Using the measurement knob, adjust the bottom scale (sucrose scale) of the measuring field to read exactly 0. Mentally note the position of the field boundary relative to the crosshairs. This is the target position of field boundary.

APPENDIX F List of students and projects under my supervision

1. Alice Stembridge – Determination of the Conductivity, Moisture Content, Water Activity and Viscosity of Model Honey Solutions
2. Evie Trolove – Refractive Index, Density and Surface Tension of Model Honey Solutions
3. Bastein Haution – Physical Properties of Honey Mixture
4. Hamish Grigg – Water Activity of Model Honey Solutions
5. Beth Te Aika – Crystallisation of Glucose in Model Honey Solutions
6. Hasnul Hizam – Measurement Techniques and Theories of Water Activity for Concentrated Sugar Solutions and Model Honey Solutions
7. Pierre-Vincent Facoëtti – Properties of Model Honey Solution
8. David Bishop – Modelling Honey Crystallisation
9. Jessica Brenton – Analysis of Glucose Crystallisation in Model Honey Solutions using Microscopy
10. Chris Ireland – Crystallisation of Glucose in Model Honey Solutions